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THE MORPHOLOGY OF MONOPETALONEMA ALCEDINIS (RUDOLPHI, 1819) (NEMATODA: FILARIOIDEA) INCLUDING ITS FIRST-STAGE LARVA[1]

ROY C. ANDERSON

Abstract

Monopetalonema alcedinis (Rudolphi, 1819) is reported in *Megaceryle a. alcyon* (Linné) (belted kingfisher) from Ontario. The new material is described and several features of this important species are pointed out, viz. the cephalic papillae are double; the male caudal alae meet on the ventral surface in front of the anus; there is a median papilla immediately in front of the anus as well as two pairs of postanal structures (? phasmids and postdeirids); the cuticle of the body is covered with cuticular bosses; the distal filament of the left spicule is alate. *M. alcedinis* is compared with the other species assigned to the genus (*arthricola*, *angustispiculum*, *upembanum*, *upembanum kilwezi*, *solitarium*) and the egg and first-stage larva are described in detail.

Introduction

The author has recently had an opportunity to study specimens of *Monopetalonema* Diesing, 1861 found by Prof. A. M. Fallis in *Megaceryle a. alcyon* (belted kingfisher) from Ontario. Although there is little doubt that these specimens refer to the genotype, *M. alcedinis* (Rudolphi, 1819), this study has revealed a wealth of important characters hitherto undescribed for this species. It is desirable, therefore, that a description of this material should be made. The author has, in collaboration with Dr. A. G. Chabaud (4), already noted in a key that the cephalic papillae are double and that this character readily separates *Monopetalonema* from the other genera of the Dicheilonematinae. Moreover, no detailed description of the first-stage larva of any member of the Dicheilonematinae has been published and, inasmuch as larval characters are of fundamental importance in the modern systematics of the filarioids, a description of the larva of *M. alcedinis* is given.

Materials and Methods

The adults were studied after clearing in glycerine (caudal and cephalic papillae), lactophenol, and beechwood creosote (internal organs). Eggs removed from the anterior parts of the uteri were studied in water or glycerine. Stained larvae were pressed from eggs after the latter had been kept

[1]Manuscript received March 16, 1959.
Contribution from the Department of Parasitology, Ontario Research Foundation, Toronto 5, Ontario.

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for a few minutes in a mixture of lactophenol and cotton blue; the lactophenol softened the shell. Many larvae were studied in various positions until a satisfactory conception of the morphology was obtained after which a generalized figure of the larva was made (Fig. 7).

Monopetalonema alcedinis (Rudolphi, 1819)

(Figs. 1–12)

Description

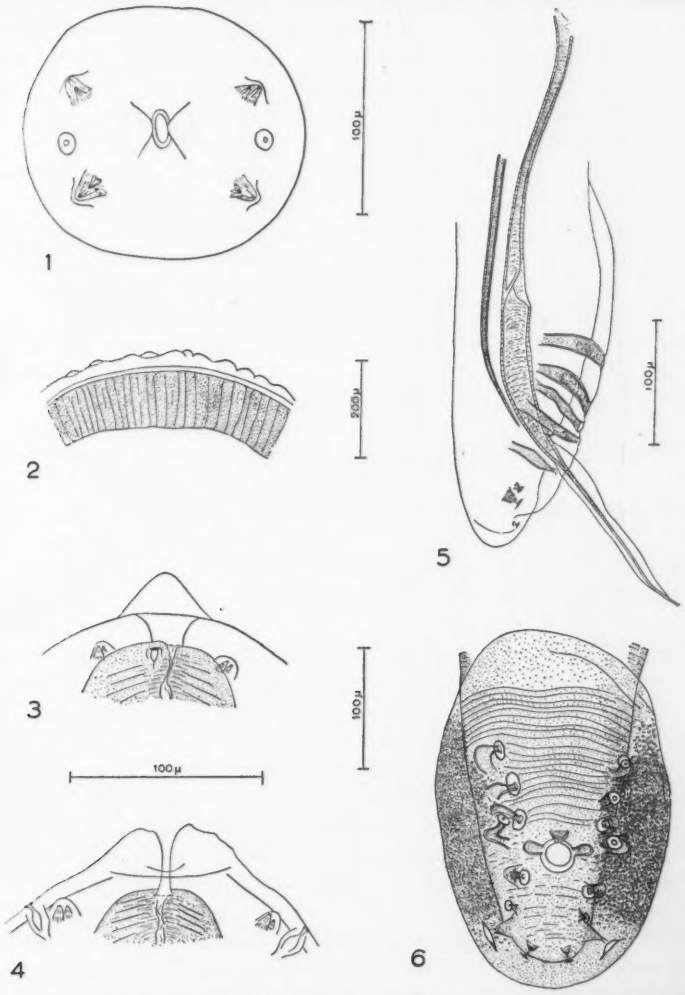
Diplotriaenidae, Dicheilonematinae, *Monopetalonema* Diesing, 1861. Cephalic papillae consisting of four double papillae of external circle. Oral opening dorsoventrally elongated, bordered laterally by pair of conical, cuticular elevations. Cuticle thick, covered with large, flat bosses most noticeable in cross section. Lateral alae absent.

Female (one specimen).—Length 243 mm. Maximum width 0.89 mm near middle of body. Nerve ring 0.26 mm from anterior extremity. Muscular oesophagus 0.39 mm, glandular 9.5 mm in length, the latter broad and dark. Vulva, a transverse slit on slight swelling 0.70 mm from anterior extremity. Caudal end blunt and rounded with pair of minute phasmids. Inconspicuous anus 0.14 mm from caudal extremity. Eggs slightly oval-shaped, thick-shelled, 43 (42–44) by 33 (32–34) μ in size.

First-stage larva (Figs. 7–12).—Length approximately 200 μ. Maximum width 11–12 μ. Nerve ring obscure, about 33 μ from anterior extremity. Oesophagus 90 μ in length, apparently narrow except for swelling of posterior part. Intestine broad and thin-walled, about 55 μ in length. Fusiform rectum about 20 μ in length. Excretory vesicle and cell prominent, 67 μ from anterior extremity. Anus exceedingly indistinct, 32 μ from caudal extremity. Cuticle with faint, transverse striations. Lateral alae present, beginning about 20 μ from anterior extremity and terminating in region of rectum. Left side of head end with pair of raised cuticular ridges in each of which there are at least four dark spots presumably papillae. Lateral to these two ridges (on the left side) is a distinct, hooked tooth. Cuticle on dorsoventral sides of anterior extremity (on each side of paired cuticular ridges) slightly thickened to form plate-like structures. Oral opening not seen. Anterior end of body surrounded by about 17 rows of minute, posteriorly directed spines, starting about 5 μ and finishing about 20 μ from anterior extremity. Tail conical, blunt, terminating in numerous delicate spines one group of which form circlet about tail. Genital primordium not detected.

Male (one specimen).—Length 63 mm. Maximum width 0.65 mm near middle of body. Muscular oesophagus 0.44 mm, glandular 9.2 mm in length. Nerve ring obscure, 0.30 mm from anterior extremity. Anus circular, surrounded by cuticular ring 100 μ from posterior extremity. Caudal alae broad and thick joining well behind termination of body as well as in front of anus. Caudal papillae projecting through thick cuticle arranged as follows: (*a*) Four pairs of elongate, preanal papillae on either side of ventrolateral surface of body, (*b*) a single median papilla immediately in front of anus, the apex

of which penetrates cuticular ring surrounding anus, (*c*) a pair of papillae on either side of anus appearing to arise from ring surrounding anus, (*d*) two pairs of papillae behind anus on ventrolateral surface, the most anterior pair elongate, the other pair minute, conical, and inconspicuous. In addition to papillae there are two pairs of phasmid-like structures on the ventrolateral surface of end of tail. Each consists of cone-shaped extension of body wall and funnel-shaped canal in cuticle communicating to exterior by fusiform pore.



FIGS. 1–6. *Monopetalonema alcedinis* (Rudolphi, 1819).
FIG. 1. Anterior end female, *en face* view. FIG. 2. Cross section through body, female.
FIG. 3. Anterior end male, lateral view. FIG. 4. Anterior end male, dorsal view.
FIG. 5. Caudal end male, lateral view. FIG. 6. Caudal end male, ventral view.

The larger, more anterior pair may represent postdeirids and the other the phasmids. Right spicule narrow and needle-shaped, 0.25 mm in length. Left spicule 0.57 mm in length, divided into tubular calomus about 0.20 mm in length and broad lamina which tapers to a narrow, alate filament.

Host.—*Megaceryle a. alcyon* (Linné) (Alcedidae).

Location.—Air sacs.

Locality.—Peterborough, Ontario.

Specimens.—Nematode Collection, Ontario Research Foundation No. 83.

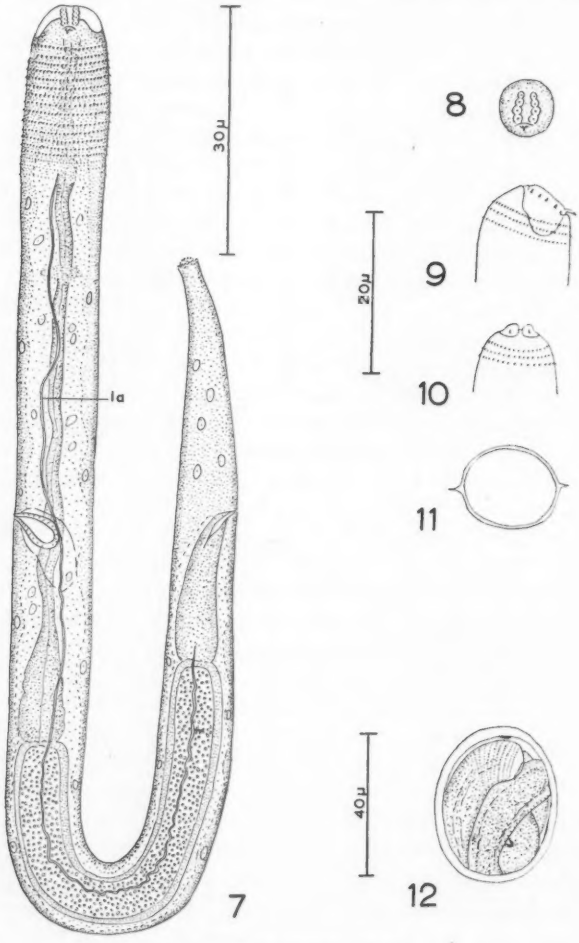


FIG. 7–12. *Monopetalonema alcedinis* (Rudolphi, 1819).

FIG. 7. Diagram of first-stage larva from egg, left lateral view. FIG. 8. Anterior end of first-stage larva, left epical view. FIG. 9. Anterior end first-stage larva, ventral view. FIG. 10. Anterior end first-stage larva, view of right side. FIG. 11. Section through body of larva showing lateral alae (*la*). FIG. 12. Larva in egg from vagina of female.

Discussion

This species has previously been reported from *Ceryle amazona*, *C. aenia*, *C. alcyon*, and *Megaceryle torquata* from South America and Egypt (2, 6, 11). Mawson (7) mentioned, but did not describe, specimens from *Megaceryle a. alcyon* from Quebec. The specimens described herein are assigned to *M. alcedinis* because they agree well with the description given by Freitas and Lent (6) and the host is similar to those parasitized in South America. It is necessary, however, to point out several features of this type species which have been overlooked by other authors.

(*a*) The four cephalic papillae noted by several authors (6, 8, 9) are double and not single as previously reported (presumably this is true for all members of the genus). In the other genera of the Dicheilonematinae there are always eight widely separated papillae of the external circle often tending to be disposed in a circle about the oral opening.

(*b*) The caudal alae meet on the ventral surface before the anus, and the cuticle on the ventral surface of the caudal end of the male is exceedingly thick and the papillae penetrate through it to the surface.

(*c*) There are, in addition to the caudal papillae reported by Freitas and Lent, a single median preanal papilla immediately in front of the anus (already noted by Mawson (7)) and two pairs of postanal structures which may be phasmids and postdeirids.

(*d*) The cuticle of the body is covered with broad flat cuticular bosses that were seen only in cross section.

(*e*) The distal filament of the left spicule is alate (this could probably not be observed if the spicule was retracted).

Besides *M. alcedinis* five species of *Monopetalonema* have been described, all but one from Alcedidae:

(*i*) *M. arthricola* (Skrjabin, 1916) (9) from *Ceryle* sp. from Paraguay is presumably distinct from *M. alcedinis* in that it possesses lateral alae and a much shorter left spicule.

(*ii*) *M. angustispiculum* (Sandground, 1933) (8) from *Ceryle lugubris guttulata* from Indo-China has, according to Sandground, small slender spicules which presumably distinguish it from *M. alcedinis*.

(*iii*) *M. upembanum* (Vuylesteke, 1953)(10) from *Megaceryle m. maxima* from the Belgian Congo is exceedingly close to *M. alcedinis* and future study may show that it is a synonym of the latter. One notes especially that the caudal alae meet before the anus and that the caudal papillae are similar to those found in the specimens described herein. The main difference is in the length of the left spicule ("0.830 mm"). Freitas and Lent (6) give a range of 0.52–0.67 mm however.

(*iv*) *M. upembanum kilwezi* (Vuylesteke, 1953)(10) also from *M. m. maxima* of the Belgian Congo is said to be distinct from the foregoing in the presence of undulating striations on the ventral surface of the caudal end of the male (also noted in the specimen from Ontario) and in the form and length of the spicules. The author suspects strongly that this subspecies is invalid. The

undulating striations on the ventral surface can be expected to be highly variable depending largely on the fixation, and the appearance of the spicules often depends on their position. The lengths of the spicules are not greatly different. Future study will have to determine if the species from Africa is distinct from *M. alcedinis* of North and South America. Boulenger (2) described as *M. physalurum* (Bremser, 1851) specimens from *Ceryle torquata* from Egypt but did not describe the spicules or caudal papillae. Freitas and Lent (6) regarded this species as a synonym of *M. alcedinis*.

(*v*) *M. solitarium* Caballero, 1948 (3) from *Myiozetetes* sp. (Tyrannidae) of Mexico, the only species reported from other than kingfishers, is known from a single female specimen. Since the apices of the peribuccal elevations are concave in lateral view and the eggs only 37 by 16 μ in size, it is presumably distinct from *M. alcedinis*.

The first-stage larva of only one species of Diplotriaenidae has been described (*Diplotriaenoides translucidus* (1) and one species of the Aproctinae (*Aprocta fiorii*)(5)). The larva of *M. alcedinis* is similar in many respects to that of *D. translucidus*. The latter is stouter, however, and lacks the two raised cuticular ridges found at the anterior end of the larva of *M. alcedinis* and the caudal extremity consists of a single circlet of spines only. The tail of the larva of *Aprocta fiorii* is exceedingly long and attenuated and no spines were noted at the anterior end.

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SEASONAL CHANGES IN THE CYTOLOGY OF THE OVARY AND OF THE PITUITARY GLAND OF THE GOLDFISH[1]

ALAN WINSTON BEACH[2]

Abstract

Goldfish ova undergo maturation from November until the following July. Seasonal changes in the maturation and resorption of follicles were correlated with changes of the area of pituitary basophils in sagittal sections. Resorbing ova are found throughout the year but are more abundant at sexual maturity and particularly prominent in ovaries of fish which fail to spawn. Resorption is associated with a marked hypertrophy of the follicular granulosa and this layer is evidently responsible for the removal of egg contents. The cytology of the developing ova and of the hypertrophied granulosa of the resorbing follicle are described. The possible source of the ovarian hormones is discussed but the present study provides no evidence for the cells responsible for their production.

Introduction

The goldfish (*Carassius auratus*) is a seasonal breeder producing eggs during the spring and early summer. It may be assumed that the ovary of the goldfish, like that of most other animals, produces hormones as well as the eggs. Both functions are probably essential to the perpetuation of the species since the ovarian endocrines are responsible for much of the reproductive physiology and behavior characteristic of the female animal. These events may be expected to be timed and controlled by gonadotropins of the pituitary gland.

At the present time there is considerable uncertainty as to the nature and cellular source of the ovarian hormones of fishes (6) as well as the ovarian–pituitary interrelationships in this group of vertebrates. It was for this reason that the present investigation was undertaken. A careful description of the seasonal changes in the cytology of ovary and pituitary might be expected to produce some suggestive evidence as to the source of the hormones. At any rate, such a study is a necessary preliminary to experimental work.

Particular attention was directed toward those follicles which, during their development, undergo atresia. These bodies are frequently conspicuous in the fish ovary and have attracted considerable attention. Some workers have suggested that they are the source of hormones (2, 3, 5). However, it is well known that the ovaries of animals contain more potential eggs than are ever matured and that, in all vertebrates, some must undergo atresia. The particular cytological development which has been described in the teleost ovary may be associated with the removal of the large amount of yolk rather than the secretion of hormones. On the other hand, both functions may be performed by the so-called "pre-ovulation corpus luteum" (6). It was hoped that a seasonal picture of the changes in these bodies correlated with the

[1]Manuscript received March 2, 1959.
Contribution from the Department of Zoology, University of British Columbia, Vancouver, Canada.

[2]Graduate student. These data are taken from a thesis presented in partial fulfillment for the M.A. degree at the University of British Columbia.

pituitary cytology would bring further information to bear on this problem and would serve as a basis for an experimental investigation of the ovarian–pituitary interrelationships in the goldfish.

Materials and Methods

Goldfish, averaging about 9 cm in fork length, were obtained during the late summer from the Goldfish Supply Co., Stouffville, Ontario, and kept in aquaria at $20° \pm 2°$ C. They were maintained under routine conditions and fed 0.15 g Pablum (Mead Johnson & Co.) per fish daily for 4 consecutive days with ground shrimp meal on the fifth day.

Samples for histological examination were preserved regularly every second week. The right ovaries were fixed in Bouin's picric acid – formol – acetic acid mixture and the left ovaries and pituitaries in 10% formalin containing mercuric chloride.

Dehydration was accomplished by Smith's (14) *n*-butyl alcohol series, which penetrates well and renders yolky tissue suitable for sectioning. The pituitary tissue, which was always left in the parasphenoid bone when removed from the head, was decalcified with 20% nitric acid prior to dehydration. Vacuum-embedding was necessary to remove gas bubbles and antemedium.

Sections were cut routinely at 10 μ. A standard number of 200 transverse serial sections of the ovary were mounted. The pituitaries were sectioned sagittally and every 10th section mounted on glass.

All of the pituitaries and most of the ovaries were stained by the PAS (periodic acid Schiff) technique (7, 8, 9, 10, 11). Some of the ovaries were stained routinely with Mayer's haemalum and eosin.

Differential counts of all the ova in arbitrarily determined stages (see below) were made on every 10th to 15th section of the ovaries. The pituitary analysis was based on a measurement of the area of basophilic staining cells in relation to the total area of the transitional lobe in the section under study. It is appreciated that the basophils produce hormones other than gonadotropins (6). Atz (1) was able to distinguish the gonadotrophs and thyrotrophs using this technique on pituitaries of *Astyanax* but our preparations showed no clear differentiation. In addition these cells produce ACTH and possibly other materials. However, the seasonally changing activity in the area probably represents, at least in part, changing activity of the gonadotrophs and was the only morphological evidence available. The relative size of the areas was determined by projecting the sections onto tracing paper, drawing the areas carefully, and measuring them with a polar planimeter.

Results

Histology of the Ovary

Oogenesis in the Goldfish

The formation of eggs in the ovary of teleosts has been frequently described (15). The germinal epithelium surrounds the gland and extends as folds into its substance. During the period of intense oogenesis young ova are pro-

liferated uniformly throughout this epithelium. Some new ova are evidently being formed at all times in the goldfish ovary but the process is in abeyance during the period of egg maturation.

For convenience the developing eggs will be divided into three groups for description: (*a*) *immature ova*—the primary oocytes prior to the appearance of yolk, (*b*) *maturing ova*—cells in which the yolk is accumulating and the nuclear membrane remains intact, (*c*) *mature ova*—eggs larger than 500 μ in diameter with much yolk, no nuclear membrane, and prominent nucleoli.

Immature Ova

The cells proliferated from the germinal epithelium may be referred to as the *primary oocytes*. Early in their enlargement, one of the neighboring germinal epithelial cells begins to divide to enclose the developing oocyte with a layer of follicular cells known as the *granulosa*. Fibroblasts from the ovary wall proliferate to form a layer around the granulosa. This is known as the theca. The processes seen in the goldfish correspond closely to those described in the bitterling by Bretschneider and de Wit (2, 3).

In newly formed follicles, the cytoplasm of the egg is clear and shows strands of material between the cell membrane and the nucleus. This stage is rather transitory. Later the cytoplasm stains with haematin, showing a strong basophilic character. The nucleoplasm of these cells is clear, almost non-staining, and contains one darkly staining giant nucleolus averaging 7 μ in diameter. It seems to be attached to the nuclear membrane. This giant nucleolus later gives rise to several small ones which are still located near the nuclear membrane (Fig. 1). These nuclear changes in the developing ova were described in an excellent way as long ago as 1891 by Calderwood (4). More recently, Yamamoto (18) has studied the origin and formation of nucleoli in the flounder, *Liopsetta*, using both cytological and cytochemical techniques.

The primary oocytes in which the giant nucleolus has given rise to several smaller ones show the first traces of an exceedingly thin zona pellucida. It is at first basophilic but becomes feulgen-positive in the next stage.

Maturing Ova

Yolk first appears in eggs of about 150 μ diameter. The primary yolk globules appear in a ring within the cytoplasm about one-quarter of the distance from the cell membrane to the nucleus (Fig. 2). The globules are at first clear and colorless but turn acidophilic and feulgen-positive by the time a second layer of yolk globules appears. Successive layers of yolk globules form centrally from the primary layer until about three layers have formed after which formation is random. The chemical nature and origin of the yolk granules have recently been described in the flounder, herring, and smelt by Yamamoto (16, 17, 19, 20, 21, 22).

The nuclear membrane shows signs of disintegration in ova of 300 to 400 μ diameter and the ova then mature very rapidly. This maturation is characterized by extensive yolk deposition accompanied by considerable increase in size, a pronounced thickening of the zona pellucida or oolema, and an

increased prominence of the follicular cells—possibly associated with the mobilization of materials for yolk formation.

The zona pellucida or oolema at this stage attains a thickness of about 9 μ and shows marked radial striations while the outer surface becomes very irregular (Fig. 3). The oolema stains feulgen-positive.

Three distinct layers are now evident in the follicle. The zona granulosa remains distinct while the connective tissue or theca layer can be differentiated into two zones, the theca interna and the theca externa. The theca externa appears as a layer of simple, non-stratified squamous epithelium. The cells are disc-shaped and attached to neighboring cells at the edges. The theca interna is similar in structure but consists of several layers. The zona granulosa is a thicker layer and is syncytial. All follicular cells stain with haematin.

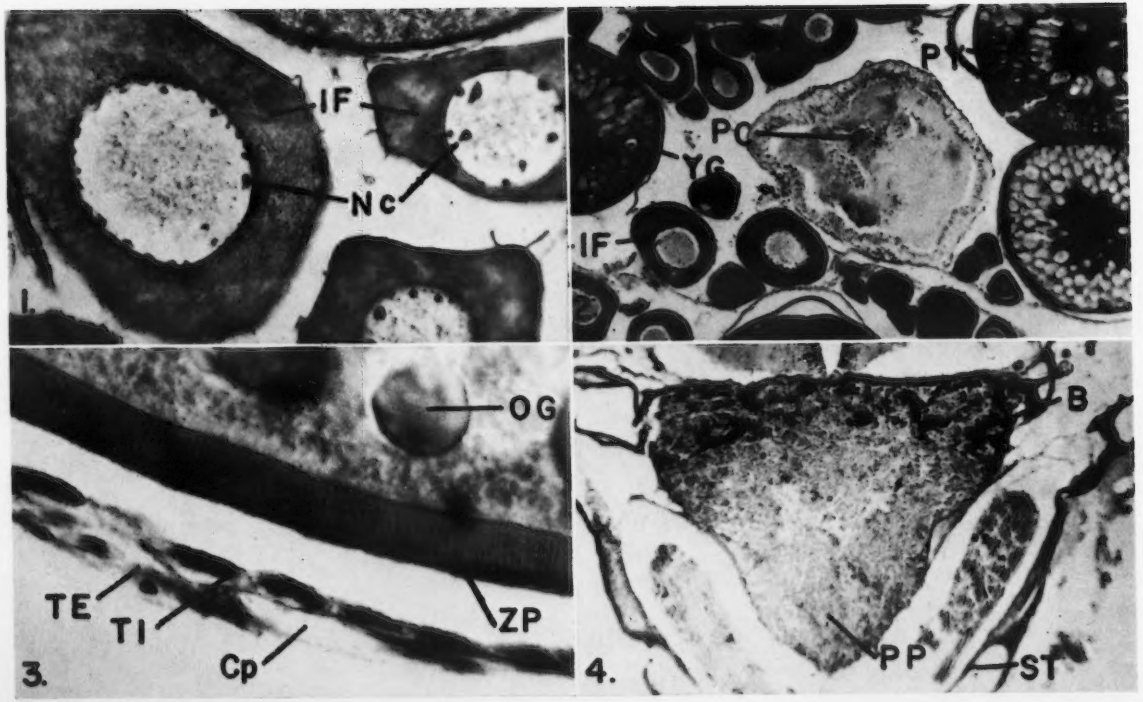
Mature Ova

Ova larger than 500μ in diameter are termed mature. The cytoplasm is filled with yolk which is more prominent peripherally. The nuclear membrane has disappeared and the nuclear material seems to be dispersed throughout the cytoplasm. Only occasionally are fragments of the nucleoli evident. The zona pellucida is prominent. The follicular sheath has not changed from the last stage. Mature ova are illustrated in Fig. 3.

Histogenesis of Follicles Undergoing Atresia

Follicles of any age and size may be resorbed. The process is probably fundamentally the same in all, but differs somewhat in appearance depending on the amount of yolk present in the resorbing egg. In fish which fail to spawn or are prevented from spawning, the amount of yolk which must be removed is maximum. The process of resorption in immature follicles presumably is rapid. The character of the zona granulosa is the most interesting feature of these follicles. It shows the first changes characteristic of the resorption process. The syncytial granulosa, which is initially almost as thin as the thecae, begins to hypertrophy and distinct cell membranes appear within it. The syncytial stage comes to an end and numerous granules appear within the cells. The function of these hypertrophied granulosa cells has not been determined. Some writers have looked upon them as phagocytic and regarded the granules as yolk material (3). It seems possible that the granules are secretory and produce the enzymes which digest the yolk, the cytoplasm, and the nucleus of the ovum. There still remains the possibility that they are hormone-producing or may have this function in addition to that of digesting the resorbing ovum.

FIG. 1. Immature follicles. ×400. Nc—nucleoli of various sizes and shapes.
FIG. 2. A *c*-stage degenerate follicle showing invasion of granulosal cells. ×100. PY—primary yolk globules; IF—immature follicles; YG—yolk globules; PC—invading granulosal cells.
FIG. 3. Outer section of a mature follicle showing zona pellucida with radial striations. 2×900. ZP—zona pellucida; TE—theca externa; TI—theca interna; Cp—capillary; OG—yolk globule.
FIG. 4. Transverse section through the posterior region of a goldfish pituitary. ×80. PP—pars posterior; B—basophils in transitional lobe; ST—sella turcica.



Beach—Can. J. Zool.

In maturing follicles with little yolk the process of atresia differs little from that described for the primary oocytes. The yolk granules disappear quickly in the early stages of resorption.

In maturing and mature follicles it is convenient to distinguish four atretic stages (*a*, *b*, *c*, and *d*) in this process, following the same sequence as was used by Bretschneider and de Wit (3) in their study of the bitterling ovary.

The a-stage of atresia (Fig. 5) is transitory and characterized by granulosal hypertrophy and the end of the syncytium. At this time granules have not appeared in the granulosal cells but they are vacuolar and clear. The nuclei

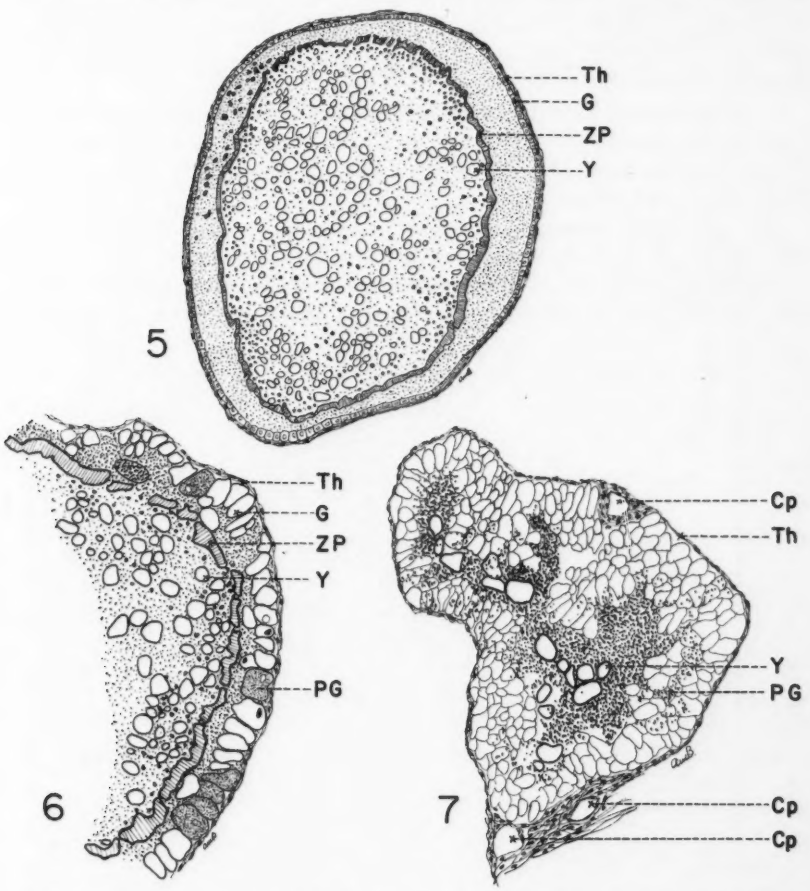


FIG. 5. An *a*-stage degenerate follicle. Th—thecal cells; G—granulosa; ZP—zona pellucida; Y—yolk.

FIG. 6. A *b*-stage degenerate follicle. Th—thecal cells; G—granulosa; ZP—zona pellucida; Y—yolk; PG—cellular granulation.

FIG. 7. A *c*-stage degenerate follicle. Cp—capillary; Th—thecal cells; Y—yolk; PG—cytoplasmic granules.

of these cells are located near the oolema and contain definite nucleoli. Colloidal fluid sometimes collects between the granulosa and oolema during this stage. The oolema shows signs of erosion, and the end of the *a*-stage is marked by its eruption.

During the *b-stage* the ruptured oolema persists and most of the egg material is removed. The thick oolema is broken in many places but the spherical form of the follicle may persist. The granulosa shows further hypertrophy than the *a*-stage (Fig. 6).

The *c-stage* shows a further thickening of the granulosa (Figs. 2, 7) so that masses of cells extend into the area formerly occupied by the egg cytoplasm. Traces of the oolema remain and a reticulum is sometimes evident in the persisting cytoplasm. The granulosal elements have all the characteristics of physiologically active cells.

In the *d-stage* the granulosa collapses to fill the space formerly occupied by the egg cytoplasm. No yolk granules are now evident and the remaining cytoplasm stains variously, frequently acidophilic on one side of the body and basophilic on the other.

The cells of the stratum granulosum in these fully differentiated resorbing follicles are cytologically characteristic. The epithelium is now tall, stratified, and columnar (Fig. 2). Their cytoplasmic granules stain acidophilically in the area adjacent to the acidophilic part of the resorbing ovum and baso-

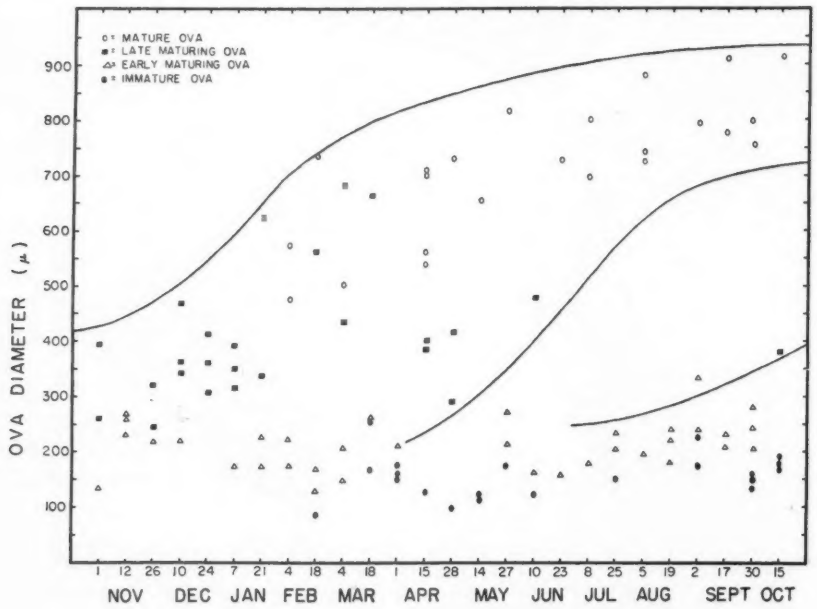

○ = MATURE OVA
■ = LATE MATURING OVA
△ = EARLY MATURING OVA
● = IMMATURE OVA
OVA DIAMETER (μ)
900
800
700
600
500
400
300
200
100
1 12 26 10 24 7 21 4 18 4 18 1 15 28 14 27 10 23 8 25 5 19 2 17 30 15
NOV DEC JAN FEB MAR APR MAY JUN JUL AUG SEPT OCT

FIG. 8. Graph showing development of follicles throughout the year.

philic in the other area. Very large "vacuoles" containing particulate matter are characteristic (Fig. 7). This may be yolk in the process of removal. The nuclei of these granulosa cells are located in the basal part of the cell near the resorbing ovum.

It is interesting to note that no hypertrophy or other changes are evident in the thecae of the goldfish. Bretschneider and de Wit (3) described marked changes in the interna of the bitterling. In the goldfish the externa becomes markedly vascularized but this seems to be the only change.

Seasonal Changes in the Ovary

Seasonal variations in the number of ova of different types are shown graphically in Figs. 9 and 10. Immature and early maturing ova are present throughout the year but mature ova are absent from October until February or

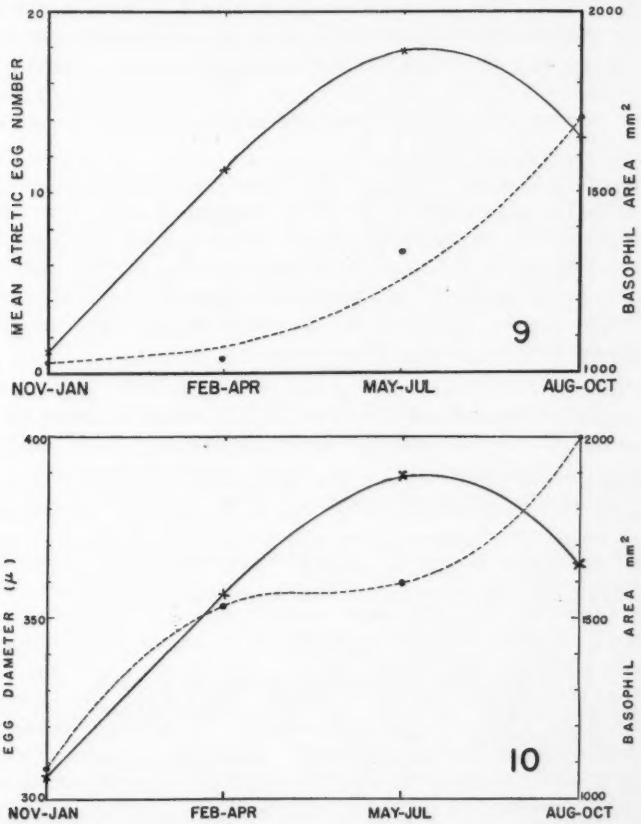


FIG. 9. Graph comparing atretic egg number and increase in basophils throughout the year. Basophils ——solid line; atretic egg number . . . dotted line.

FIG. 10. Graph comparing ova growth and increase in basophils throughout the year. Basophils ——solid line, egg diameter . . . dotted line.

March. The period of egg maturation, during which yolk is accumulating within the cytoplasm, begins during late September or October and extends through February or March when mature ova start to form rapidly. This seems to be rather a characteristic sequence of events in teleosts which breed annually in the spring or summer. It has been described in trout by Scott (12).

There is evidently considerable variation in the time of yolk formation in different individuals and it is impossible to know from this type of a study just how long the process lasts in any one egg. In the stock of fish under examination the process was evident over a maximum period of 7 months (October to June). The distribution of points in Fig. 8 indicates that the events last 4 to 5 months in any individual.

The goldfish is known to have a rather long spawning period (15). The prolonged period during which mature ova are found in the ovaries is, therefore, to be expected. However, it seems likely that if these fish had been able to spawn, a sharp break in the number of mature ova would have been observed in the late summer. Goldfish do not usually spawn in aquaria. Figure 9, showing the rapid development of resorbing follicles after July, is indicative of the fact that spawning time had passed and the mature ova were being removed.

It is interesting that the resorption of follicles may be found at any time in the year (Fig. 9). The number, however, increases as the animals begin to mature during April. It would be valuable to know what Fig. 9 would have looked like if the fish had been able to spawn. As it is, the large numbers formed in the summer and autumn represent ova which could not be removed in normal spawning and provide little suggestive evidence for any endocrinological functions which these bodies may have.

Correlation Between the Histological Changes in the Ovary and the Pituitary

Egg diameter was chosen as an index of maturation and the area of PAS positive cells (Fig. 4) as criterion of gonadotropic activity. Figure 10 shows an evident correlation between ovary and pituitary activity as measured in this way. The statistical details given in Table I show that the correlation was positive and significant at each of the four seasons tested.

TABLE I

Correlations of pituitary basophils with egg diameters

Season	Mean basophil area, mm^2	Mean egg diam., μ	r	t	P	N
Nov.–Jan.	1064.4	307.4	+.476	2.54	0.02	24
Feb.–Apr.	1567.3	352.8	+.562	3.25	0.001	30
May–July	1899.0	360.0	+.832	6.00	0.001	18
Aug.–Oct.	1648.0	400.7	+.574	3.66	0.001	29

TABLE II

Correlations of pituitary basophils with atretic egg numbers

Season	Mean basophil area, mm²	Mean atretic egg number	r	t	P	N
Nov.–Jan.	1064.4	0.59	+.444	2.32	0.02	24
Feb.–Apr.	1567.3	0.63	−.771	6.41	0.001	30
May–July	1899.0	6.97	−.218	0.90	0.4	18
Aug.–Oct.	1648.0	14.54	+0.63	0.33	0.75	29

The area of pituitary basophils was also studied in relation to follicular resorption. The results are given in Fig. 9 and Table II. It is evident that the area of pituitary basophils reaches a maximum just prior to the time of greatest atresia of eggs. It would seem, therefore, that the pituitary influences atresia of eggs and once having served its functions, begins to decline. These quantitative data are in line with the findings presented above.

Discussion

The structure of the ovary of several teleosts has been carefully described. In addition to the earlier literature, which has been recently reviewed (5, 6), a series of articles by Yamamoto (16–23) has added considerable cytological detail especially concerning the histochemistry of the yolk granules and the nucleolus. No detailed descriptions of the structure of the goldfish ovary has, however, come to my attention. The only paper on this organ is a general one dealing with its development (15).

The structure of the goldfish ovary is, in general, similar to that of other teleosts. New eggs proliferate from the germinal epithelium throughout the year but the period of maturation during which the yolk accumulates extends from October through to the early summer of the next year. Mature ova are abundant in the ovaries of laboratory-reared fish throughout the spring and summer but are evidently resorbed in the autumn when spawning has not occurred.

The resorbing follicles are of particular interest as a possible source of ovarian hormones. During egg resorption the stratum granulosum proliferates extensively to form a large mass which obviously performs some important function. This layer of the follicle is intimately associated with yolk deposition and yolk removal in all vertebrates which produce yolky eggs. These may be its only functions. However, in the mammals, this same layer hypertrophies after ovulation to produce the corpus luteum which is known to produce the hormone progesterone. The phylogeny of this important chemical material has not been described. In fish the granulosa may or may not hypertrophy after ovulation (5, 6) and it has not yet been determined whether this layer (either as a pre- or a post-ovulation corpus luteum) produces an endocrine secretion. Estrogen, which is evidently produced by the fish ovary, is probably produced by the theca. The problem has been reviewed (6).

The present study shows that an hypertrophy of the granulosa may occur in the immature as well as in the maturing and the mature ova. The process becomes more marked as the eggs begin to mature. This might argue for either a role in hormone production or a removal of surplus eggs for which there is perhaps insufficient nutriment or inadequate circulation for maturation. This problem might not be resolved by histological methods. That the granulosa is the active organ in yolk removal is obvious. It seems likely that these cells produce enzymes which digest the yolk and prepare it for absorption into the blood. Some writers have, however, described the process as one of phagocytosis (3).

It has been recognized for many years that the pituitary of the teleost controls the activities of the gonads. Scruggs (13) described seasonal changes in the cytology of the hypophysis of the goldfish. In this paper the size of the basophilic area of the gonad has been correlated with growth and resorption of the eggs. The problem of the nature of the teleost gonadotropin(s) and the mechanism by which they control the cytological changes in the ovary remains to be investigated.

Acknowledgments

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REPRODUCTION IN THE EUROPEAN HARE IN SOUTHERN ONTARIO[1]

J. K. REYNOLDS[2] AND R. H. STINSON[3]

Abstract

Certain aspects of the reproductive biology and growth of the European hare, *Lepus europaeus hybridus* Desmarest, were studied in 1949–52 in southwestern Ontario. The breeding season began in January and declined in August, reaching its height between the second week of February and the third week of June when all females examined were pregnant. The litter size varied from one to four; an average of 1.6 foetuses occurred in the early litters (January–March) and 3.8 in later litters (April–June). One young raised in captivity grew to adult weight (4100 g) in 6 months.

Introduction

The European hare, or "jack rabbit" as it is known in Ontario, has become an important member of the fauna since its introduction from Europe in 1912. Dymond (2) has described its introduction into southwestern Ontario and Reynolds (9) has given an account of its subsequent spread until now it occupies almost the whole of southern Ontario up to the Pre-Cambrian shield. The hare has been identified by Peterson and Reynolds (8) as *Lepus europaeus hybridus* Desmarest, the subspecies which in Europe ranges eastward from eastern Germany into central Russia (4).

Kolosov (6) has made a detailed study of reproduction in this hare in western Russia but, except for a report by Silver (11) and scattered observations, little is known of its life history in North America.

Collection of Specimens

The study was carried out over the years 1949–52 when hares were collected during summer field work on watershed surveys, and during the winter from organized drives by sportsmen. Animals were taken in every month of the year although the drives in the months of December to March accounted for 75% of the total. With exception of a few hares from neighboring counties, all were obtained from the counties of Elgin, Middlesex, Oxford, Perth, and Grey.

Results

Reproductive Organs

Except for their larger size the external and internal organs do not differ appreciably from those of the domestic rabbit as described by Craigie (1). Due to the difficulty in obtaining specimens in the late summer and fall as well as the problem of distinguishing between young and adult at this time, changes in size of the ovary and testis were not followed through the year.

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Contribution from the Department of Zoology, University of Western Ontario, based upon a thesis submitted in partial fulfillment of the requirements for the Ph.D. degree. Financial assistance was received from the Research Council of Ontario.
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The average dimensions of the ovary in non-pregnant females in November 1949 (12 females) were 15×7×5 mm, in December (19 females) 22×11×8 mm, and in January 1950 (24 females) 24×14×10 mm. These figures may reflect both the growth of young to sexual maturity and the seasonal onset of the reproductive cycle. Kolosov (6) does not describe any seasonal fluctuation in ovary size, and states that the size and weight of the organ depend very little on its functional condition.

Externally the ovarian follicles and corpora lutea appear as translucent dots on the surface. The corpora lutea may measure as much as 6 mm in diameter at which time they often occupy a large percentage of the total volume of the ovary.

In the male the testes appear below the surface of the abdominal musculature after the embryo has reached a length of 80 to 85 mm, and descend to the scrotum when the hare is presumably sexually mature. As in other lagomorphs the inguinal canal remains open throughout life and the testes may be withdrawn through it. It appeared from smears and histological examination that scrotal testes contained live spermatozoa while abdominal testes lacked any spermatozoa. However, the position of the testes after death could not always be relied upon as a criterion of sexual activity. On one occasion when a wounded animal was first examined both testes were observed to be in the scrotum. After the animal had been dead for a short time they were found to be abdominal. Schwartz (10) found that a similar movement might occur in the cottontail rabbit after shooting or trapping. The average dimensions of the testes of 91 hares taken in December to March and 23 hares taken in May to August during 1949–50 were, length with one standard deviation 56.3±3.3 mm and thickness at center 18.8±1.6 mm.

The male:female sex ratio in 264 hares taken in the field from February 1950 to April 1951 was 1.06:1.0. In 142 embryos of determinable sex it was 1.09:1.0. Kolosov (6) found a ratio of 1.05:1.0 in adult hares.

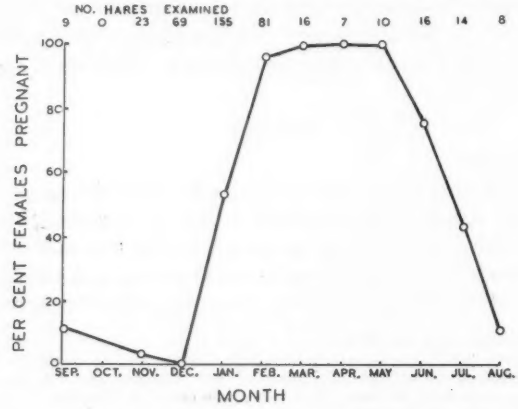


FIG. 1. Incidence of pregnancy among 408 females of *Lepus europaeus hybridus* examined in 1949–52.

Breeding Season

In the period 1949–52, 408 females were examined for the presence of embryos. By November the young of the year could not be distinguished from the adults and hence all ages are present in the data for the winter months (Fig. 1). When the beginning of the breeding season in January was analyzed in more detail it was found that the number of pregnant females rose as follows: by the end of the first week 8%, by the second week 41%, by the third week 61%, and by the end of the fourth week 83%. Between the second week of February and the third week of June all females examined were pregnant. On this basis the breeding season in Ontario may begin earlier than it does in Europe. Lonnberg (7) stated that in Sweden breeding begins in February and extends into September, while Kolosov (6) found that the 100% level of pregnancy was not reached until May in the Caucasus region.

Litter Size

No more than four embryos were found at any time (Table I), although Dymond (3) referred to a litter of six at Meaford, Ontario, and Dr. C. H. D. Clarke of the Ontario Department of Lands and Forests examined a hare containing 10 foetuses near London in April 1933.

TABLE I

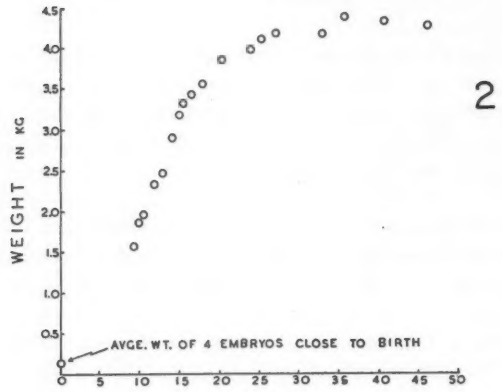
Variation of litter size in 211 pregnant females of *Lepus europaeus hybridus* in period 1949–52

No. embryos	Month												
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
1	68	36									1		105
2	12	28	9		1								50
3	2	13	6		1	1	3	1					27
4		1	1	7	4	11	3	1	1				29
Mean size	1.2	1.7	2.5	4.0	3.5	3.9	3.5	3.5	4.0		1.0		1.9

The seasonal change in litter size is similar to that found by Kolosov (6) but there was little evidence of the high prenatal mortality he reported. He stated that 19% of the winter and 25% of the autumn embryos underwent atrophy and absorption but in the present study only five resorptions were found, four of these occurring in the winter months.

Behavior and Growth

As in other hares, the young at birth are fully furred with their eyes open. The following note was made of the behavior of two young: from each of two pregnant females secured on March 25, 1950, a male foetus was removed within a few minutes after the death of the adult. Both young gave convulsive gasps and began to breathe within a few seconds of being freed from the embryonic membranes. They were placed on a wet sack on the snow in the sun where they crawled about for 2 hours exposed to a light breeze with the air temperature 8° C. Both animals reacted to handling by grunting, attempting to bite, and scrambling out of reach. They were fed a warmed mixture of equal parts of cows' milk and water. During the first day neither



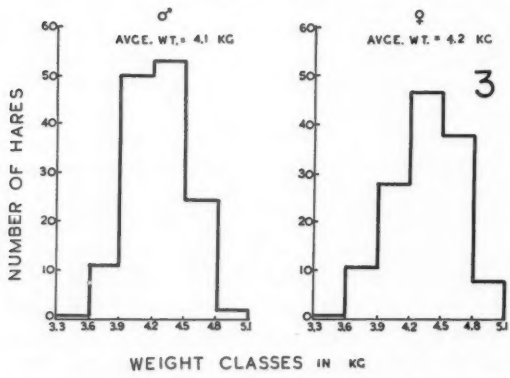


FIG. 2. Growth rate of one male of *Lepus europaeus hybridus* received at age of 9 weeks.

FIG. 3. Weights of 136 males and 128 females of *Lepus europaeus hybridus* collected from February to April 1950 and 1951.

moved about very much and when they did a crawling motion was used with one foot moving at a time. By the third day both were sitting up and actively hopping across the floor like adults, frequently following a human walking across the room. Although a sugar mixture was later added to the milk, they survived for only 5 days.

Although the European hare uses a rough open form for a nest only two young were found in the field. These weighed 140 and 142 g. As Hediger (5) found, great difficulty was experienced in keeping young in captivity owing to the highly excitable nature of the animal and the constant need for cleanliness. Only one was raised to an adult, a young male caught by the sons of Dr. A. M. Fallis shortly after it was born. It was received by us in October, 1950, when it was about 9 weeks old, and kept under observation until June, 1951, when its age was 46 weeks (Fig. 2). The average adult weight of 4180 g given by Peterson and Reynolds (8) was reached in 6 months.

If the data of Fig. 2 are representative of development in this hare the population in terms of weight must be largely adult by February since breeding declined rapidly after July (Fig. 1). The weight distributions shown in Fig. 3 for hares taken in the months of February to April are indicative then of the range of size in adult males and females. The female weight includes that of the embryos and lactating tissue.

Discussion

From an examination of the ovaries the first pregnancy of the season could be recognized but not subsequent ones. It was not possible then to determine the number of litters in a season, nor could it be shown that the increase in average litter size (in Table I) occurred in successive litters of the same animal. However, Hediger (5) has reported that in captivity the female of *L. europaeus* in Switzerland has a gestation period of about 42 days, and that it has been observed to mate on the day of parturition. From the length of the breeding period in the present study, and particularly the fact that for a period of 18 weeks from February to June all females were pregnant, there would appear to be time for at least three litters and possibly four. A rough calculation of the reproductive potential then may be made on the basis of three litters. If the average size of the first is 1.6 (average of January, February, and March, in Table I), the second 3.8 (April, May), and the third 3.8 (June, July, August), the total number of young per season would be nine.

A comparison may again be made with the findings of Kolosov (6). In the northern part of its range (55° N. lat.) in western U.S.S.R. *L. europaeus* has two litters a year with an average total production of seven young per female. In the Caucasus steppes, however, an area at a similar latitude to southern Ontario (44° N.), the female may have four litters a year with a total of 10 young.

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THE CUTICLE OF THE TWO-SPOTTED SPIDER MITE, TETRANYCHUS TELARIUS (LINNAEUS) (ACARINA : TETRANYCHIDAE)[1]

K. E. GIBBS AND F. O. MORRISON

Abstract

The cuticle of *T. telarius* (L.) examined by light and electron microscopy and subjected to tests for chitin and lipids is shown to be a thin layer with a pattern of external ridges. It is about 1.25 microns thick measured in the troughs and twice that thickness measured at the ridges. The ridges average from 1 to 1.6 μ apart. There is no tectocuticle but an outside lipoid layer and a dark-staining non-chitinous epicuticular layer from 0.1 to 0.2 μ thick. A double-layered inner procuticle is present. The outer layer of the procuticle remains unstained in contrast to the dark-staining inner layer. The inside surface of the cuticle bears elevations or ridges opposite the external troughs. The epicuticle only is shed at molting. It bears the branched setae and over the eyes is striated, the ridges being about 0.14 microns apart. Either one or both layers of the procuticle contain chitin.

Introduction

There is a considerable body of information on insect cuticle in the published literature (14, 20, 21, 22, 23) but arachnid and particularly acarine cuticle has been comparatively little investigated. Browning (3) and Sewell (16) studied spider cuticle and Lees (9) that of ticks, Vitzthum (19) reviewed all the previous work on mite cuticle and in so doing pointed out that Thor's 1904 paper (18) remains the authoritative work on the integument of the prostigmatic group. Jones (7) concluded that the cuticle of mites was very similar to that of ticks and insects.

The possibility that there might be some basic differences in the outer coverings of mites and insects and that such differences might help to explain the differential contact effects of certain chemicals on the two groups engendered the present investigation.

Materials and Methods

Mites of the species *Tetranychus telarius* (Linnaeus) as designated by Boudreaux (2) were examined in surface view and cross section by means of optical and electron microscopes. Sections were prepared from adult females and from quiescent female deutonymphs, and exuviae of the latter were also examined. Electron micrographs were taken on 2-in. by 2-in. lantern slide plates of normal contrast in an RCA Model EMT electron microscope at a magnification of 3000 diameters and enlarged photographically as desired, all measurements being made from enlarged prints.

[1]Manuscript received September 5, 1958, and as revised, May 13, 1959.
Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Que. Macdonald College Journal Series No. 429. Based on a thesis submitted by the senior author to the Faculty of Graduate Studies and Research of McGill University in partial fulfillment of the requirements for the degree of Master of Science.

Mites to be sectioned for electron microscope study were fixed in either 1% osmium tetroxide (10) or in Gilson's fluid (6) and embedded in a mixture of one part methyl methacrylate and two parts *n*-butyl methacrylate to which 2% of 2,4-dichlorbenzoyl peroxide, as catalyst, had been added. The methacrylate blocks were hardened in gelatin capsules by exposure to ultraviolet light at a temperature of 47° C. Within 8–12 hours the plastic was sufficiently polymerized to be cut.

The sections were made with a Spencer rotary microtome with a wedge modification of the type designed by Pease and Baker (12). With the wedge in, the machine could be set to advance from 0.2 to 0.1 μ on each turn. The sections were mounted on a formvar film on appropriate grids. When the mounts were dry they were immersed for 20 minutes in toluene to remove the embedding medium, then redried and placed in the microscope for examination.

Sheets of cuticle or exuviae were not sectioned but examined directly in surface view. The pieces were picked up with a syringe from water on which they floated, and deposited in water droplets on the grid on a microscope slide. The water was removed by means of filter paper strips and by evaporation while the integument was oriented and flattened on the mount by the use of a fine pin.

Material for the light microscope was prepared by standard embedding and sectioning techniques and stained with haematoxylin or with Masson's trichrome stain (11).

Chemical tests carried out included the chitosan tests as employed by Campbell (4), treatment with concentrated hydrochloric acid, and treatment with cold and boiling chloroform for the removal of lipoids.

Results and Discussion

The elastic, transparent, colorless cuticle of *T. telarius* observed under a light microscope shows patterns of numerous fine parallel lines or striations such as occur on most tetranychid mites. Under the electron microscope the striations appear to be cuticular thickenings from 1.0 to 1.6 μ apart. In cross section (Fig. 2) they show up as ridges which in some views (Figs. 3 and 4) appear to be knobbed or expanded at their apices. The cuticle in the troughs between the ridges averages 1.25 μ in thickness, while measured at the apices of the ridges it is 2.5 μ thick. That stretching might alter these thicknesses is suggested by the report (13) that in actively feeding forms the striations appeared less distinct or broken.

Blauvelt (1) reported that in haematoxylin-stained sections of *T. telarius* under an optical microscope the cuticle showed up as a finely ridged dark-staining outer layer and a paler inner layer. The writers using Masson's trichome stain and the optical microscope observed a dark-staining outer layer, a pale middle layer, and an inner green layer. Osmium-tetroxide-fixed sections in the electron microscope showed three distinct layers also (Fig. 2). The outer and inner layers appeared dark either due to a different consistency from the middle layer or to a different affinity for the osmium tetroxide.

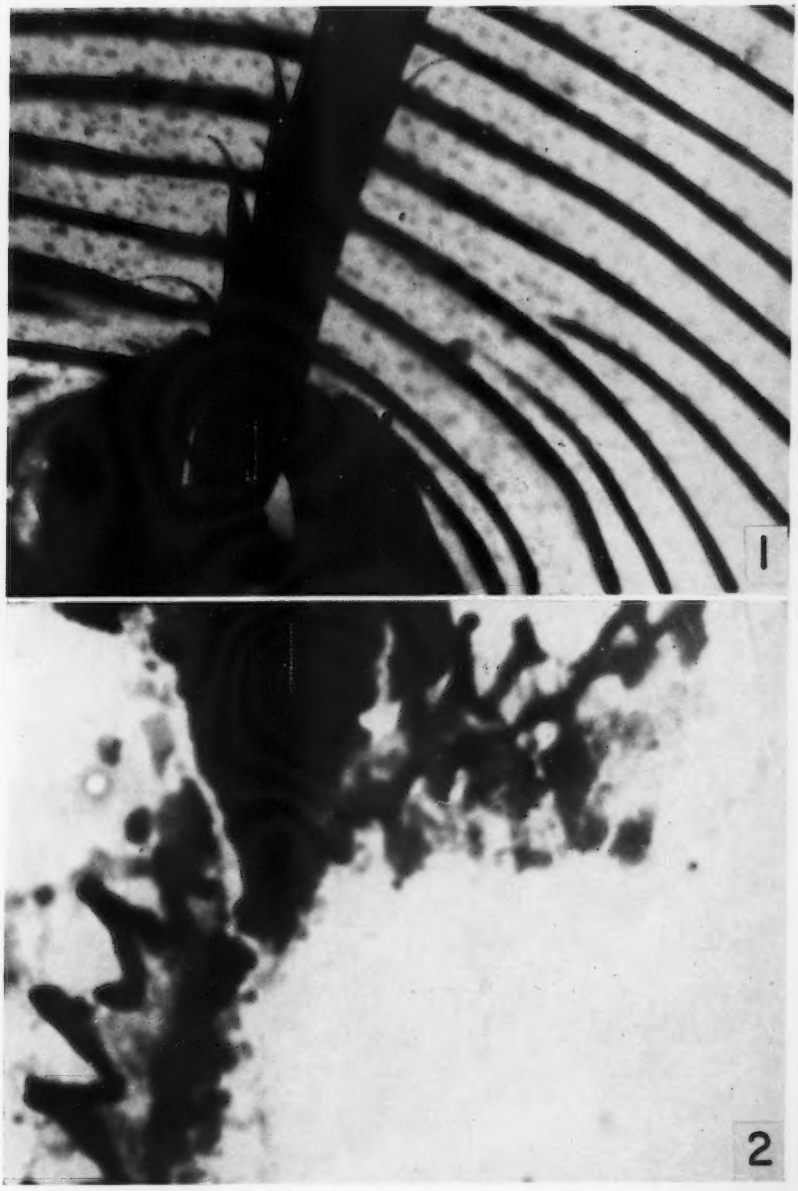
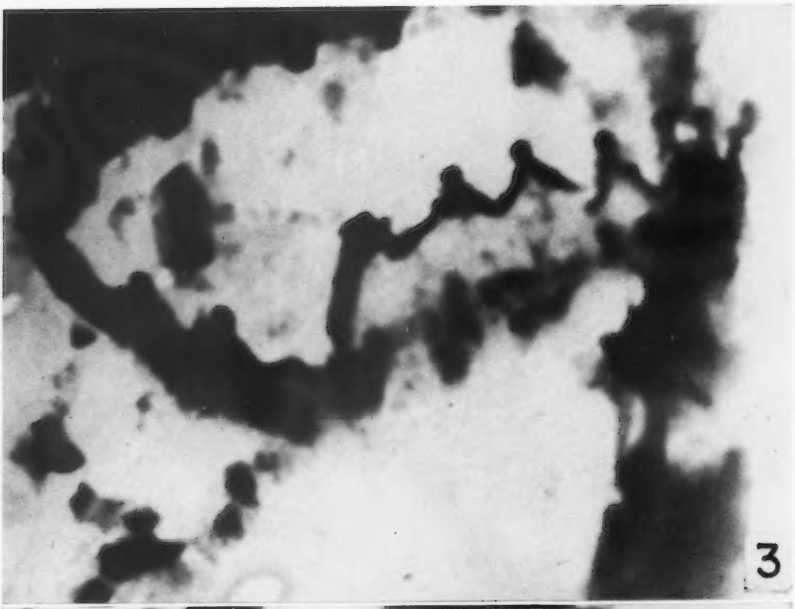


FIG. 1. Exuviae of a female deutonymph showing base of a seta. Surface view. ×14,000

FIG. 2. Section of the integument of an adult mite fixed overnight in osmium tetroxide. ×14,000



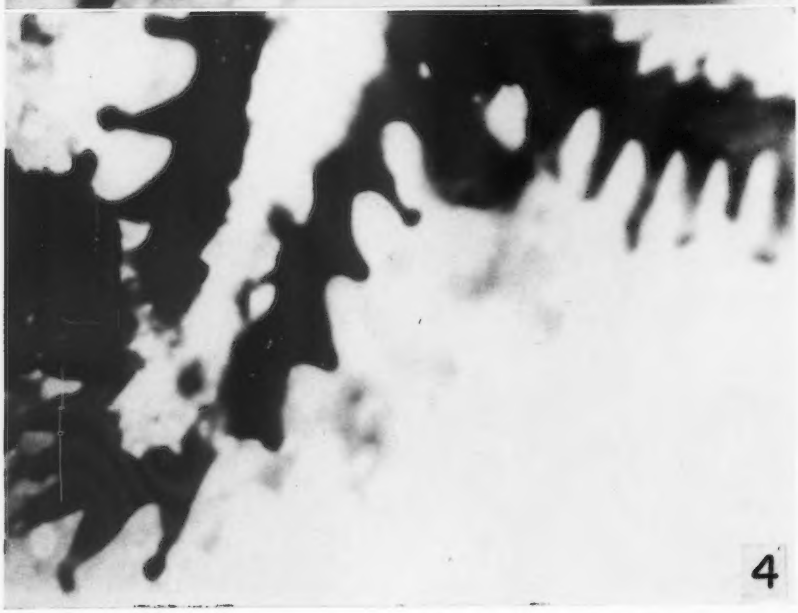


FIG. 3. Section of the integument of an adult mite fixed overnight in osmium tetroxide. ×14,000
FIG. 4. Section of the integument of an adult mite fixed in Gilson's. ×14,000

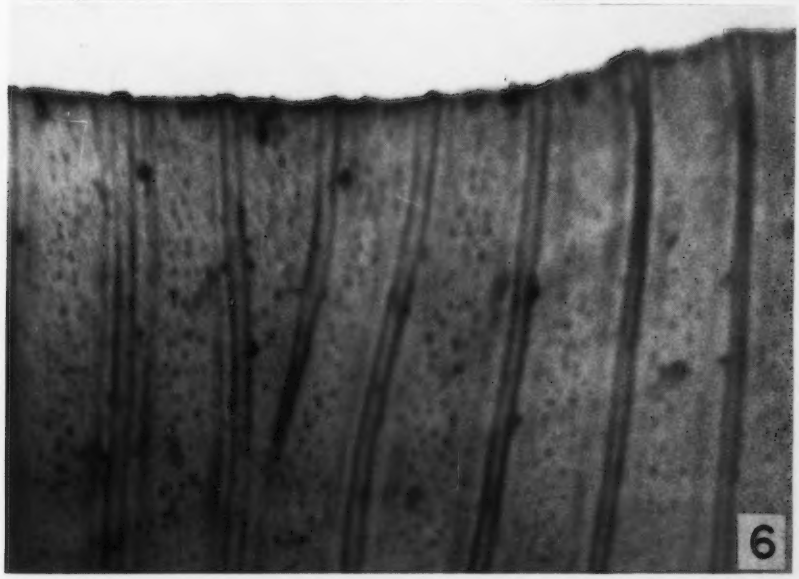


FIG. 5. Section of a quiescent female deutonymph fixed in Gilson's. The cast skin (on the right) has torn away from new integument (left) when the film broke under the microscope. ×14,000

FIG. 6. Cast skin of a female deutonymph showing flattened appearance of the ridges where the cast skin split at ecdysis. Surface view. ×14,000

Epicuticle

The dark-staining outermost layer of the cuticle appears to be shed intact at the time of molting and hence to form the exuviae. Figure 5 is a picture of a portion of the deutonymph integument at the time of molting. The cast skin has been pulled away from the newly formed integument by a break in the formvar membrane on which it was mounted for examination. The form and ridged nature of the exuviae correspond to that of the epicuticular layer as seen in Fig. 2. The new epicuticle has the same form. In surface view (Fig. 6) the ridges of the exuviae often appear to have flattened out along the dorsal molting split.

Exuviae heated in potassium hydroxide solution to 160° C (4) dissolved. After treatment at lower temperatures no positive reaction for chitosan appeared.

On this evidence the denser outermost layer of the cuticle is here being termed the non-chitinous epicuticle since it most closely resembles the layer to which Richards (14) applies that name in his studies of insect cuticle. The outside cuticular layer may not, however, be the same structure in all mites. Jones (7) reported a similar situation in the British harvest mite, *Trombicula autumnalis* Shaw, in that only the epicuticle remained unaffected by the molting fluid and was hence cast off, but in other species Jones found the exuviae to be formed of exo- and epi-cuticle. Kirshnan (8) reported the epicuticle of the scorpion *Palamneus swammerdami* Simon to be resistant to the hot potassium hydroxide treatment and to give a positive chitosan test.

If our interpretation is right the epicuticle of *Tetranychus telarius* is from one-ninth to one-fifth the thickness of procuticle. The ratio of epicuticle to procuticle, as calculated from published figures, is about 1/20 in *Periplaneta americana* (L.) adults (15), 1/60 for *Sarcophaga falculata* Pand. larvae (22),* and 1/24 in the sclerites of the spider *Tegenaria domestica* (L.) (16).

The epicuticle of many insects is itself a multilayered structure (14) having at least an inner cuticulin layer overlaid by a polyphenol layer, overlaid in turn by a wax layer and a tectocuticle or cement layer. The polyphenol layer in insects is demonstrated by abrading or dissolving off the wax layer, then subjecting the exposed polyphenols to an ammoniacal silver nitrate solution which they reduce producing a dark color. *Tetranychus telarius* treated with cold or boiling chloroform and then with the ammoniacal silver nitrate solution exhibited a slight darkening in the area of the setae and mouth parts. This was evident under the light microscope and an irregular darkening can be seen in the electron micrographs (Fig. 8). This indicates a weak argentaffin reaction as compared to that reported for other arachnids. The polyphenol layer would not appear to be a continuous one. The existence of a discrete layer has been questioned even in insects (15).

*The figures cited by Wigglesworth (22) are those of Dennell, R. (1946). A study of an insect cuticle; the larval cuticle of *Sarcophaga falculata* Pand. (Diptera). Proc. Roy. Soc. B, 133, 348–373.

Exuviae of *T. telarius* treated with hot or cold chloroform lose their hydrophobic nature. Figure 7 shows a cast after 24 hours of treatment with cold chloroform. The structure is less distinct than that of untreated material (Fig. 1) and the presence of numerous minute dark spots suggests that the effect of the solvent has not been uniform. These observations, together with the necessity for treatment with a fat solvent before darkening on the application of ammoniacal silver nitrate can be demonstrated, suggest the presence of a lipoid layer such as occurs in insect epicuticle (9, 22). However, since concentrated hydrochloric acid destroys the entire cuticle this lipoid layer cannot be of the highly resistant type described by Dennell and Malek (5) for *Rhodnius* and *Periplaneta*, or by Sewell (16) for *Tegenaria*.

No cement layer or tectocuticle appeared to be present in the cuticles examined. This layer has been reported to be absent in certain mites (7, 19) but present in other mites (19), some ticks (9), and one spider (16).

The exuviae (epicuticle) bear numerous setae and are peculiarly modified over the region of the eyes. The setae (Figs. 1 and 10) are set in thick rings and bear triangular, pointed, scale-like projections about $1\frac{1}{2}$ microns long irregularly spaced along the shaft.

T. telarius has four eyes on the dorsum of the propodosoma. These are marked by concentrations of red pigment granules. The epicuticular covering of these eyes shows extremely fine striations (Fig. 9). In surface view they appear as some 80 to 90 fine parallel lines about 0.14 microns apart. The striations of the smaller eye on the same side are at an angle of about 60 degrees to those of the larger eye. It seems safe to assume that these lines represent a grid of cuticular ridges although we were unable to get cross sections of these organs to prove this.

Procuticle

The two inner layers of the cuticle appear to constitute the procuticle as it occurs in other arthropods. The outermost of these layers, which did not stain with osmium tetroxide or with Masson's stain, extends into the undulations of the epicuticle but is relatively even on the inner surface (Figs. 2 and 3). Its non-staining, flexible, elastic nature makes its exact homology with the layer usually termed the exocuticle questionable.

The inner layer stands out distinctly because of its affinity for the stains but shows no tendency to separate physically from the outer layer. Its staining reaction resembles that described (16) for the endocuticle of *Tegenaria*. In cross section the inside border of the cuticle shows short projections (some blunt, some sharp) (Fig. 4) occurring at regular intervals opposite the external troughs of the epicuticle. This arrangement suggests an accordion-like pleating of the whole cuticle but might equally as well be the result of a honeycomb-like structure in which hypodermal cells lodge. Should the latter speculation be right one would have to postulate a greater elasticity on the part of the inner layers as compared to the epicuticle to allow for cuticular stretching and flattening (13) of the external surface on body distention.

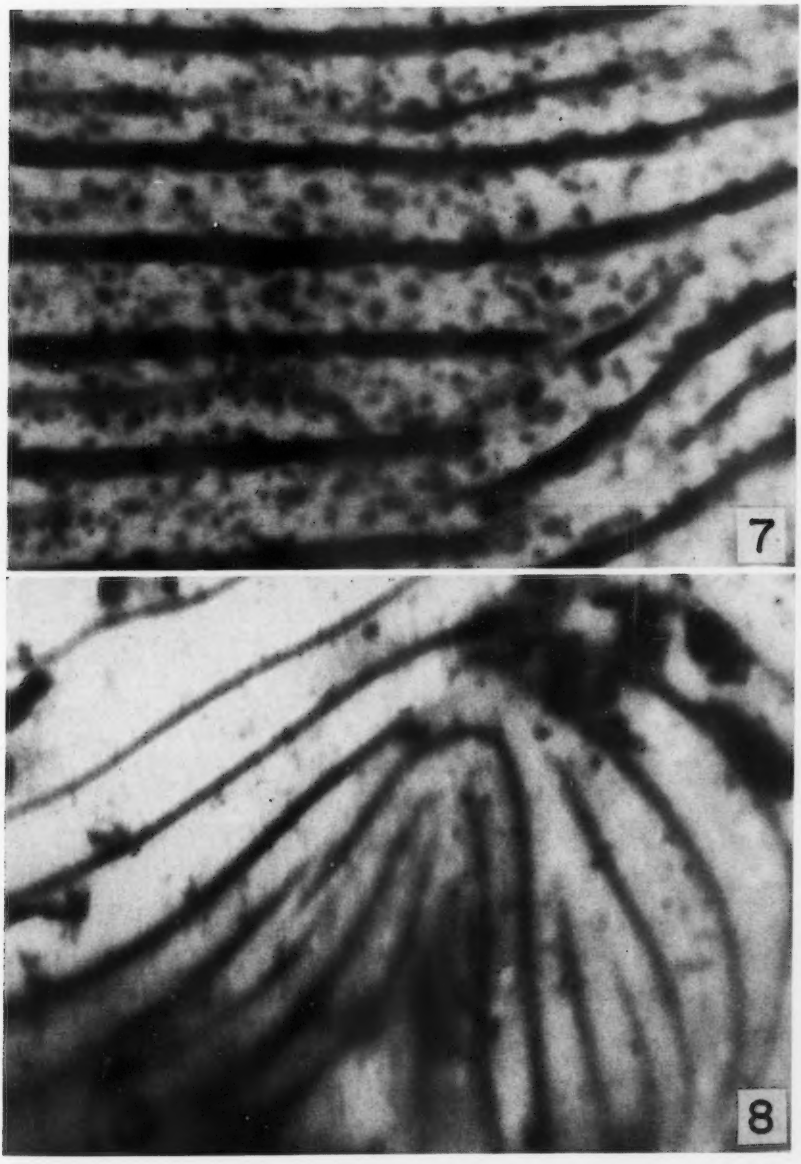


FIG. 7. Exuviae of female deutonymph after 24 hours in cold chloroform. ×14,000
FIG. 8. Exuviae of female deutonymph after 5 minutes in boiling chloroform. The irregular black spots are due to contamination in the microscope. ×14,000

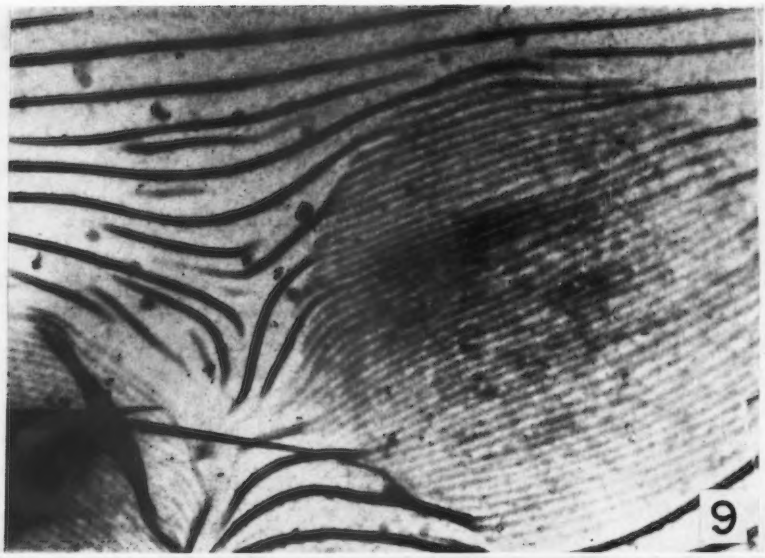


FIG. 9. Exuviae of a female deutonymph showing the surface view of the eyes. ×8400
FIG. 10. Setal shafts from exuviae of a female deutonymph. ×14,000

Treatment of whole mites with hot concentrated potassium hydroxide leaves a thin cuticular covering which retains the shape of the mite and gives a positive test for chitosan. Therefore either one or both layers of the procuticle contain chitin.

Cross sections failed to reveal any sign of pore canals through any of the cuticular layers. Thor (18) claimed that such canals were usually absent in mites of this group. Canals of a diameter as great as 0.6 μ have, however, been observed in the integument of the *Halarchne otariae* Steding (17).

Hypodermis

Neither optically examined sections nor electron micrographs gave any clear-cut picture of the hypodermal cells.

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THE SPECIES OF ISOCAPNIA BANKS (INSECTA, PLECOPTERA, NEMOURIDAE)[1]

WILLIAM E. RICKER[2]

Abstract

The stonefly genus *Isocapnia* has 10 known species in the North American cordillera, 1 in Japan, and 1 in coastal Siberia. Of the American species, *fraseri*, *hyalita*, *missourii*, and *mogila* are here described as new, as is the Siberian species *kudia*. Unusual or perhaps unique among stoneflies is the occurrence of dwarf males, having rudimentary wings, along with normal males, in the Japanese and in several American species.

Introduction and Acknowledgments

Specimens used in this study have been borrowed from or examined in the Canadian National Collection, Science Service, Ottawa (CNC), the Cornell University Collection (CUC), the United States National Museum (USNM), the Illinois Natural History Survey (INHS), the Harvard College Museum of Comparative Zoology (MCZ), the collection of the Department of Zoology of Montana State College, Bozeman (MSC), and that of Mr. S. G. Jewett, Jr., Portland, Oregon (SGJ). Mr. Jewett very kindly drew to my attention the USNM specimen described as *I. kudia*, and also the specimens of the new species *mogila* which he had himself collected. Specimens not otherwise designated are or have been in my own collection. Mr. R. A. Hays, Dr. F. Neave, and Messrs. Angus, Eric, John, and Karl Ricker have given assistance in taking specimens.

Isocapnia Banks 1938

1938. *Isocapnia* Banks, Psyche, **45**(1), 73. Genotype: *Arsapnia grandis* Banks 1908.
1942. *Isocapnia* Frison, Pan-Pacific Entomologist, **18**(2), 68.
1946. *Isocapnia* Hanson, Am. Midland Naturalist, **35**(1), 235.

Having only a short history as a named entity, the genus *Isocapnia* has roused interest partly by its rareness and partly because of the large size of several species, as compared with most capniines. It is known to occur in the cordilleran region of North America from coastal Alaska and central British Columbia south to Colorado, Utah, and central California; also in Japan and coastal Siberia.

Since J. F. Hanson's detailed illustrations of capniine adult external anatomy became available in 1946, there has been no need to confuse *Isocapnia* with other genera: the thoracic sterna are particularly distinctive. The known nymphs are unusual, among stoneflies, in having long swimming hairs along the cerci.

Dwarf Specimens

The most interesting structural feature of *Isocapnia* is the occurrence of dwarf males in certain species. Such males are most easily distinguished by their very reduced wings, commonly only 1 or 2 mm long, but some species

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also exhibit differences in the form of the genital structures. In species which have long-winged males as well as dwarf ones, the former show no tendency toward wing reduction or intergradation with the dwarf forms in this respect, though the body sizes may overlap.

The occurrence of dwarf males is as follows:

Only dwarf males known: *missourii*.
Both dwarf and normal males: *agassizi*, *hyalita*, *fraseri*, *integra*, *japonica*, *spenceri*.
Only normal males known: *abbreviata*, *crinita*, *grandis*, *mogila*.

Most species have been so little collected that both kinds of males may yet be found for them. However, Mr. S. G. Jewett, Jr., and I have both searched unsuccessfully for dwarf *grandis* in several places where long-winged specimens were numerous, so that dwarfing in *grandis* must be quite an uncommon condition if it occurs at all. Even in places where dwarf *spenceri* was common, dwarf *grandis* failed to turn up. On the other hand, dwarfing need not occur everywhere that a species occurs: *Isocapnia agassizi*, which has numerous dwarf individuals on the Fraser River, yielded none at all in several fairly large collections at Cameron Creek (though dwarf *spenceri* was found at the latter locality), and there are none in two Oregon collections. Dwarfing of females has been tentatively recognized in two specimens of *I. agassizi*, but in these the wings exhibited no undue reduction in size.

Brachyptery, by itself, is exceptional though not rare among Plecoptera; it usually affects males much more severely than females, but the latter are fairly often partially affected, and occasionally as much as the males. Some species of *Alloperla*, *Eucapnopsis*, etc., are completely long-winged in some habitats, while both sexes are partly or completely short-winged in others. The unusual feature of the brachyptery in *Isocapnia* is the occurrence of extremely short-winged and completely long-winged specimens in the same population *without intergradation*. Also, brachyptery in these *Isocapnia* is associated with a general dwarfing of the body of a considerable portion of the male population. It is possible that the dwarfs are precocious year-old mature specimens in populations where many males and almost all females mature at 2 years. This suggestion is prompted by the analogy with several species of salmon of the genus *Oncorhynchus*, in which precocious maturity of part of the stock is common among males (the so-called "jacks"), and is rare among the females. This is true of *O. nerka*, *O. kisutch*, and *O. tshawytscha*.

Species Groups

Structurally the American species of *Isocapnia* are divisible into three groups, as follows:

The *grandis* group—*agassizi* Ricker, *crinita* Needham and Claassen, *grandis* Banks. Costal cross veins numerous, four to eight before the end of *Sc*, two to four beyond; ♂ supra-anal process long and slender; ♂ 9th tergite without a posterior process; female subgenital plate with a central recessed sclerotized portion set off by large weakly sclerotized areas on either side;

nymphs with short body hair (longest hairs of the 9th tergite 1/8 to 1/4 as long as the tergite, in different species); dried exuviae with cerci almost parallel throughout their length.

The *hyalita* group—*fraseri* Ricker, *hyalita* Ricker, *integra* Hanson, *missourii* Ricker, *spenceri* Ricker: costal cross veins one to five before the end of *Sc*, none to two beyond; ♂ supra-anal process long, slender or expanded; ♂ 9th tergite with a raised bilobed or cleft posterior process; female subgenital plate entire, being even with the sides of the 8th sternite or recessed to a level somewhat anterior to it; nymphs densely covered with long hair (longest hairs of the 9th tergite 1/3 to 1/2 as long as the tergite); dried exuviae with cerci strongly curved laterally in their outer half.

The *abbreviata* group—*abbreviata* Frison, *mogila* Ricker: small; costal cross veins three to four before end of *Sc*, one beyond; male supra-anal process short, tapering, slightly to strongly hooked at the tip; female subgenital plate similar to the *grandis* group, but the median "tongue" broader and less recessed; nymphs unknown.

Of the Asiatic species, *I. japonica* Kohno is a small species from Japan, which can be included in our *hyalita* group. The other is a large new species, *Isocapnia kudia*. It is reminiscent of *I. grandis* in general appearance, but it has fewer costal cross veins than do members of the *grandis* group, and the female 8th sternite is unlike any American species (Fig. 21). The male is unknown.

Key to Adults of American Species of *Isocapnia*

1*a*. Length to tip of wings, ♂ 6–9 mm, ♀ 8–11 mm. Supra-anal process slender and short (Fig. 23); ♀ subgenital plate with a *broad* central sclerotized region (sometimes containing an unsclerotized spot) (Fig. 24); Oregon and California, two rare species..... 2

1*b*. Length to tip of wings, ♂ 9–16 mm (except "dwarf" males), ♀ 10–20 mm. Supra-anal process slender and long (Fig. 3), or shorter and stout (in *spenceri*); subgenital plate entire, or with a *narrow* central sclerotized tongue (Fig. 1)..... 3

2*a*. Veins of the forewing very irregular (Figs. 25, 26); male with a sharp thorn-like process on the basal plate of the supra-anal apparatus anterior to the supra-anal process (Fig. 23); lobe of the 9th sternite rounded behind (Fig. 27); ♀ subgenital plate with a central unsclerotized spot in the median tongue (Fig. 24)..... *mogila*

2*b*. Venation regular or nearly so; basal plate of the supra-anal apparatus with a rounded hump dorsally; lobe of the 9th sternite triangular, its hind margin broad and slightly to markedly excavated; the tongue of the ♀ subgenital plate lacking a central unsclerotized spot..... *abbreviata*

3*a*. Costal cross veins one to four (rarely five) before the end of *Sc*, and none to two (almost always one) beyond it; ♂ 9th tergite with a prominent posterior notched or bilobed process (Fig. 8); ♀ subgenital plate lacking the median tongue described below..... 4

3*b*. Costal cross veins four to eight before end of *Sc*, two to four beyond it; no raised process on ♂ 9th tergite (Fig. 3); ♀ subgenital plate with a median recessed sclerotized tongue set off by an unsclerotized notch on either side (Fig. 1)..... 8

4*a*. Wing membrane light tan in color; ♂ supra-anal process broad in side view—at least half as broad as long; ♀ subgenital plate strongly recessed; coastal plain and valleys, California to British Columbia..... *spenceri*

4*b*. Wing membrane either blackish brown or nearly clear (dark tan in *integra*); greatest width of the ♂ supra-anal process not more than 1/10 of its length; ♀ subgenital plate varies from moderately recessed (Fig. 16) to not at all recessed (Fig. 12)..... 5

5*a*. Costal cross veins usually four or five before the tip of *Sc*; ♂ supra-anal process stout, strongly curved (Fig. 14); ♀ subgenital plate moderately recessed near the middle (Fig. 16); Montana..... *hyalita*

5*b*. Costal cross veins usually three or fewer before the tip of *Sc*; ♂ supra-anal process less curved, almost straight in its outer half (Fig. 13); ♀ subgenital plate less recessed (Fig. 15) 6

6*a*. Supra-anal process uniformly slender from the base to near the apex (Fig. 13); southern Montana and probably northern Utah..... *missourii*

6*b*. Supra-anal process, in side view, thickest at or below the middle (Figs. 8, 9)..... 7

7*a*. Wing membrane blackish brown; lobe of ♂ 9th sternite often but not always narrow (Fig. 5); ♂ supra-anal process thickest at the middle; Fraser River, B.C..... *fraseri*

7*b*. Wing membrane dark tan color especially in the ♀; lobe of ♂ 9th sternite moderately broad; ♂ supra-anal process thickest about 1/3 way from the base (Fig. 9); mountains of southwestern Alberta..... *integra*

8*a*. ♂ supra-anal process elongate S-shaped in side view; ♀ subgenital plate with a short central tongue, uniformly and darkly sclerotized; southern Alaska to northern California, and inland..... *grandis*

8*b*. ♂ supra-anal process curved upward and slightly forward (Fig. 3), not S-shaped; ♀ subgenital plate with a longer central tongue, which is distinctly more heavily sclerotized along its side margins (Fig. 1)..... 9

9*a*. Supra-anal process with a small but definite hook at the tip, as seen in side view (Fig. 3); Colorado to Montana..... *crinita*

9*b*. Supra-anal process without any definite hook, more slender than in *crinita*; coastal region of Washington and southern British Columbia..... *agassizi*

List and Descriptions of Species

Isocapnia abbreviata Frison 1942

1942. *Isocapnia abbreviata* Frison, Pan-Pacific Entomologist, **18**, 71–72, Fig. 17. Male.
1954. *Isocapnia abbreviata* Jewett, J. Fisheries Research Board Can. **11**, 548, Fig. 8. Female.

This species is known from two localities in Oregon (Oak Creek, Benton Co., and the mouth of the Rogue River) and one in California (Waddell Creek, Scranton). It is smaller and more delicate than *mogila*; the ♂ supra-anal process is not so thick and has the terminal hook only slightly indicated, sometimes almost indistinguishable.

Isocapnia agassizi Ricker 1943

1943. *Isocapnia agassizi* Ricker, in part, Indiana Univ. Publs. Sci. Ser. No. 12, 90, Figs. 58, 62, 65 (*not* Figs. 63, 66). Male.
1942. *Isocapnia* sp. (discussed under *crinita*,) Frison, Pan-Pacific Entomologist, **18**, 68. (Figure 14*b* illustrates a ♂ *agassizi* of the type series.)

Female: Length of body, 9–13 mm; to tip of wings, 12–17 mm. The subgenital plate has a central, more sclerotized strip which terminates slightly anterior to the hind margin of the segment; the sides of this strip darker than the central portion (Fig. 1).

Nymph: Numerous exuviae taken at Agassiz indicate a nymph similar in general to that of *grandis*, but with shorter body hair (longest hairs on tergite 9 about 1/8 of the length of the tergite). The ♂ has a supra-anal process sheath very similar to *integra* (shown in Fig. 20), but a little less heavy.

The holotype of *I. agassizi* is a dwarf ♂. Of the mixed group described as *agassizi* in 1943, taken by the Fraser River at Agassiz, B.C., April 24, 1938, I have re-examined and determined as true *agassizi* the following: 6 dwarf ♂ paratypes; 1 long-winged ♂ paratype; 1 ♀, mentioned in footnote 12 to the description of *agassizi* but not included in the type series.

Other specimens of *agassizi* are available as follows:

BRITISH COLUMBIA

Fraser River at Agassiz-Rosedale Bridge and the abandoned ferry landing (type locality). W. E. Ricker. April 17, 1957, 18 dwarf ♂, 8 long-winged ♂, 1 dwarf ♀, 2 long-winged ♀; April 24, 1957, 22 dwarf ♂, 12 long-winged ♂, 15 ♀; May 1, 1957, 48 dwarf ♂, 15 long-winged ♂, 11 ♀; May 17, 1957, 2 dwarf ♂, 1 dwarf ♀.

Duncan, Vancouver Island. G. W. Taylor. March 28, 1906, 1 long-winged ♂ (CNC).

Cameron Creek above Cameron Lake, Vancouver Island. W. E. Ricker. May 9, 1954, 11 long-winged ♂, 4 ♀, 4 exuviae; June 19, 1955, 1 long-winged ♂, 2 ♀, 2 exuviae; April 10, 1956, 1 ♀; April 15, 1956, 6 long-winged ♂, 2 ♀, 1 exuvia; April 9, 1959, 1 ♀.

Chehailus River near Harrison Mills. W. E. Ricker. April 17, 1958, 1 ♀.

Rapids of Harrison River near Harrison Mills. W. E. Ricker. April 17, 1958, 1 ♀.

Sumas River at the highway bridge, near Chilliwack. W. E. Ricker. May 6, 1937, one ♀ (listed as *I. grandis* in 1943).

OREGON

Willamette River at Harrisburg. S. G. Jewett, Jr. March 4, 1948, 3 long-winged ♂, 1 ♀ (SGJ).

Santiam River near Jefferson, Marion Co. S. G. Jewett, Jr. March 4, 1948, 19 long-winged ♂, 3 ♀ (SGJ).

WASHINGTON

Snoqualmie. Gertrude Hoppe. May 7, 1932, 1 long-winged ♂ (INHS).

North Branch of Nooksack River, elevation 2007 ft. W. E. Ricker. May 23, 1937, 2 ♀ (listed as *grandis* in 1943).

This species emerges from the Fraser in numbers comparable to *I. fraseri*. It has also been taken on a number of smaller streams in the coastal region from southern British Columbia to the Willamette valley of Oregon. See *I. crinita* for a comparison with that closely allied species.

Isocapnia crinita (Needham and Claassen 1925)

1925. *Capnia crinita* Needham and Claassen, Monograph Plecoptera, pp. 269, 357.

1942. *Isocapnia crinita* Frison, Pan-Pacific Entomologist, **18**, 69, Fig. 14*a*—this figure of the ♀ holotype does not show detail and is misleading.

1942. *Isocapnia* sp. (discussed under *crinita*), Frison, Pan-Pacific Entomologist, **18**, 70, Fig. 16.

1943. *Isocapnia crinita* Hanson, Bull. Brooklyn Entomol. Soc. **38**, 160. Suggests probable synonymy with *grandis* Banks.

Length of body: male 9–10 mm, ♀ 12–14 mm; to tip of wings: ♂ 11–12 mm, ♀ 14–16 mm. Costal cross veins six to nine before the end of *Sc*, two to four beyond it. Usually one to three apical cross veins beyond the cord between branches of *R* or *M*, and occasionally some fusion of these veins. Color in alcohol, brown with black markings, in life solid black. Body and appendages profusely hairy, the hairs mostly erect or nearly so. Head mostly dark, with a lighter area along the frontal suture and near the compound eyes. Antennae brown. Pronotum almost equal-sided, with broadly rounded corners; a broad clear-brown marginal area completely surrounds a central area reticulately marked with black. Meso- and meta-thorax and all legs brown.

Male (not previously described): Abdomen and cerci brown, except as follows: a narrow black stripe along the sides of the tergites, broader and heavier on the 9th; sternites 4–8 have a lateral longitudinal stripe, not too distinct on the more anterior segments; the tergites are narrowly margined with black next to the front margin, rather indistinctly so on the anterior few. Across the middle of each tergite there is a transverse row of six muscle attachment marks; similar marks are present in two irregular longitudinal series on each sternite.

Ninth tergite with an hourglass-shaped median mark; 9th sternite (Fig. 7) sclerotized narrowly along the anterior margin, and broadly so posteriorly, but with a triangular non-sclerotized area between these; ventral lobe almost circular except for the narrow stalk, with very profuse, short, closely appressed hairs. Tenth tergite cleft dorsally, the posterior angle of the cleft filled by the broad triangular basal portion of the supra-anal apparatus; the supra-anal process curves gently upward and forward, with a very short blunt tip tilted abruptly forward (Fig. 3).

Female: A dorsal unsclerotized stripe on tergites 2–8; the lateral pigmented stripe of the sternites definitely present only on 9 and 8; an anterior heavily sclerotized area is present on all sternites, but it is broadly interrupted at the mid-line; on sternites 1–6 a narrow longitudinal series of irregular dots or short bars is present on either side of the mid-line and just off the margin; sternite 7 with round non-sclerotized areas as in Fig. 2.

Subgenital plate of the 8th sternite recessed (i.e., situated anterior to the level of the lateral margins of the sternite), its central sclerotized portion with margins more strongly sclerotized (Fig. 2), but the pigment does not quite reach the hind margin of the plate.

Nymph: A ♂ exuvia taken with a pair of adults on the Gunnison River closely resembles the exuvia of *agassizi*, as does a ♀ exuvia taken with adults on the Logan River. The tip of the lacinia is a broad, somewhat rounded, chisel-like blade, but in lateral view it appears composed of six to eight parallel strips or ridges, and there is a slight emargination at the tip between one or two of these. On the inner margin there is a series of strong hairs. The sheath of the ♂ supra-anal process shows the forward curvature exhibited by the adult (Fig. 20). Body hair is short.

The name *crinita* has long been an "orphan" because of the poor condition of the type and the lack of paired specimens. Through the courtesy of Dr. Henry Dietrich I have been able to study the type (the cleared abdomen), while Needham and Claassen figured its wings. The wings place the species in the *grandis* group, while the rather narrow central tongue of the subgenital plate, shape of the muscle scars, and pigmentation of sternites 8 and 9, resemble the specimen described above much more than they do *grandis*. Hanson, after examining the type, rightly concluded that it resembled *grandis* rather than any other species then known; however, he had not seen any other female specimen of the *crinita–agassizi* type.

I. crinita is so closely allied to *agassizi* that the usefulness of the latter name might be questioned. However, all interior male specimens differ from *agassizi* in having a somewhat shorter and stouter supra-anal process, with a definite hook at the tip—the last is barely suggested in *agassizi*. Females are scarcely separable structurally, although in *crinita* they average somewhat larger in size.

Material was examined as follows:

COLORADO

Gunnison River at Gunnison. W. E. Ricker. April 18, 1954, 1 ♂, 1 ♀, 1 ♂ exuvia.

MONTANA

Montana Experimental Station, Bozeman. R. A. Coley. May 12, 1917, 1 ♀ (holotype—CUC).

UTAH

Diamond Creek, north of Thistle. W. E. Ricker. April 19, 1954, 1 ♂, old and dried when found.

Logan Canyon. G. F. Knowlton and F. C. Harmston. April 20, 1957, 1 ♂ (INHS—illustrated by Frison, 1942, Fig. 16).

Ogden River, Weber Co. McTanner. April 17, 1937, No. 47, 1 ♀ (INHS).

Blacksmith Fork, Logan River, Cache Co. S. G. Jewett, Jr. April 24, 1955, 2 ♂, 1 ♀, 1 exuvia (SGJ).

Blacksmith Fork, Logan River, Cache Co. A. R. Gaufin. April 22, 1955, 1 ♀ (ARG).

Isocapnia ***fraseri*** new species

1952. *Isocapnia agassizi* Ricker, in part (not the holotype). The *fraseri* specimens wrongly included in the type series of *agassizi* include the ♀ allotype, 2 ♀ paratypes, and 3 dwarf ♂ paratypes, all from Agassiz.

Length of body, dwarf ♂ 5–6 mm, long-winged ♂ 6–7 mm, ♀ 8–9 mm; to tips of wings, long-winged ♂ 9 mm, ♀ 10–13 mm. Wings lightly infuscated with grey, appearing brown in alcohol specimens; two to five costal cross veins before the end of *Sc*, none to two beyond it.

Male: Abdominal tergites with six dorsal muscle spots and a lateral callous; 9th segment quite dark laterally. Tergite 9 with a broad black band anteriorly which comes to a sharp (less often blunt) backward-pointing tip in the mid-line; posterior margin elevated and produced into a raised process shallowly notched in the mid-line (Fig. 4). Sternite 9 with the basal lobe variable in size (Figs. 5, 6), and attached by either a broad or rather narrow petiole. Supra-anal process slender, practically straight, tapering near the tip (Fig. 8).

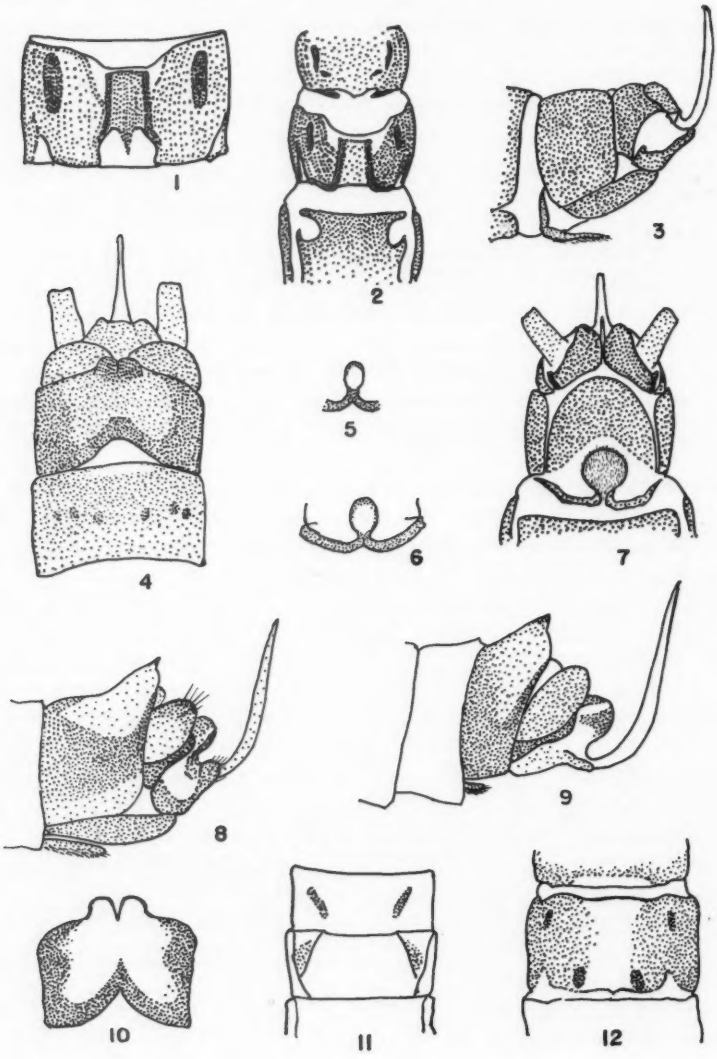
Female: Central part of 8th sternite yellow, hairless; the position of its hind margin varies from almost flush with the sides of the segment to considerably recessed, but the sclerotized lip of the plate is continuous across the segment—*not* set off by a membranous area at each side (Fig. 11).

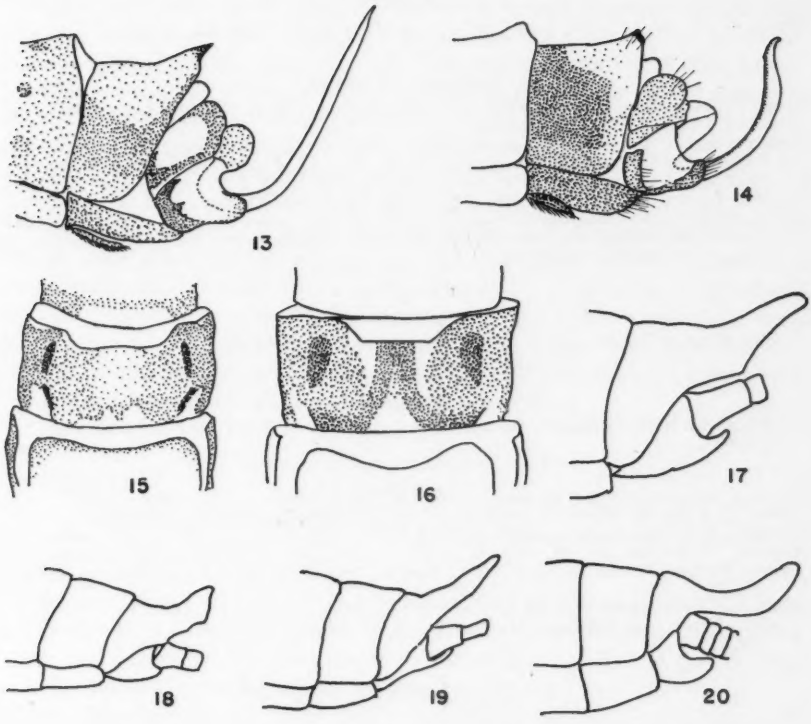
Nymph: Figure 19 shows the straight, irregularly-tapering tip of the supra-anal sheath of the mature ♂ nymph, drawn from an exuvia. The body is hairy, as in *spenceri*.

Holotype ♂ (long-winged), allotype ♀, Fraser River at Agassiz–Rosedale bridge. Angus and W. E. Ricker. April 17, 1957. Placed in the INHS.

Paratypes: same place and date as holotype, 60 dwarf ♂, 6 long-winged ♂, 11 ♀; same place, April 24, 1957, 22 dwarf ♂, 4 long-winged ♂, 14 ♀; May 1, 1957, 1 dwarf ♂, 2 ♀; March 14, 1958, 1 dwarf ♂. Paratypes have been deposited in the CNC, USNM, and SGJ collections.

This species is closely allied to *Isocapnia missourii* and *I. integra*. Differences are discussed under those species. Two specimens were obtained at Agassiz in which the process of the 9th tergite is deeply notched (Fig. 10); these might possibly represent an undescribed species.





Isocapnia grandis (Banks)

1908. *Arsapnia grandis* Banks, Can. Entomologist, **37**, 329.
1925. *Capnia grandis* Needham and Claassen, Monograph Plecoptera, pp. 259, 387. Male.
1938. *Isocapnia grandis* Banks, Psyche, **45**, 73.
1942. *Isocapnia grandis* Frison, Pan-Pacific Entomologist, **68**, 69. The male from Victoria, B.C., is selected as lectotype.
1943. *Isocapnia grandis* Ricker, Indiana Univ. Publs. Sci. Ser. No. 12, 89, Figs. 56, 57, 59, 60, 61, 64. Male and nymph.
1943. *Isocapnia grandis* Hanson, Bull. Brooklyn Entomol. Soc. **38**, 158, Figs. 3–5. Male, female.
1937. *Capnia fumigata* Claassen, Can. Entomologist, **69**, 79. Male, female.
1942. *Isocapnia fumigata* Frison, Pan-Pacific Entomologist, **18**, 70, Figs. 12, 15. Male, female.
1938. *Isocapnia fumosa* Banks, Psyche, **45**, 74, Fig. 3. Female.

Figure 17 shows the lateral outline of the supra-anal sheath of the mature male nymph, drawn from an exuvia; the slight downward curvature is distinctive. The nymph is of the "smooth" type, but its body hair is heavier than in *crinita*. *I. grandis* is the largest and most frequently collected species of *Isocapnia*, probably also the most numerous. The specimens listed below have all been examined or re-examined by me.

ALASKA

Wiseman. M. W. Johnson. June 2, 1948, 2 ♀; June 10–13, 1948, ♀.

BRITISH COLUMBIA

Cameron Creek above Cameron Lake, Vancouver Island. W. E. Ricker. May 9, 1954, 1 ♀; April 15, 1956, 1 ♀, 2 ♂ exuviae.

Haslam Creek at Cassidy, south of Nanaimo, Vancouver Island. John, Karl, and William Ricker. March 19, 1952, 1 exuvia; March 26, 1952, 13 ♂, 4 ♀, 5 exuviae; April 5, 1952, 19 ♂, 1 ♀, 4 exuviae; April 24, 1952, 2 ♂; April 10, 1955, 2 ♀.

Victoria, Vancouver Island. April 12, 1907. ♂ (holotype—MCZ).

Specimens from the following British Columbia localities were reported in detail in 1943: Agassiz; Chilliwack River at Vedder Crossing; Duncan; Fraser River at Agassiz ferry; Frost Creek above Cultus Lake.

CALIFORNIA

Feather River at Chester. W. E. Ricker. August 23, 1955, dried ♂.

MONTANA

West Gallatin River at Shedd's Bridge, Gallatin Co. R. A. Hays. June 15, 1951, 1 ♂, 1 ♀ (MSC).

OREGON

Alsea. J. E. Davis. March 21, 1929, ♂, 2 ♀ (types of *fumigata* Claassen—CUC).

Cayuse. S. G. Jewett, Jr. April 15, 1952, 2 ♂, 5 ♀ (SGJ).

Eugene. B. Malkin. March, 1947, ♀ (SGJ).

Oregon National Forest, Herman. A. C. Burrill. April 18, 1920, ♀ (type of *fumosa* Banks—MCZ).

Umatilla River at Pendleton. S. G. Jewett, Jr. April 15, 1952, 4 ♂ (SGJ).

Santiam River near Jefferson, Marion Co. S. G. Jewett, Jr. March 4, 1948, 13 ♂, 6 ♀ (SGJ).

Isocapnia ***hyalita*** new species

Length of body, long-winged ♂ 13 mm, dwarf ♂ 9–11 mm, ♀ 13–16 mm; to tip of wings, long-winged ♂ 14 mm; ♀ 15–18 mm. Wings lightly infuscated with grey. Costal cross veins four to five before the subcosta, one or two beyond.

Male: Ninth tergite with a rather small terminal process, consisting of two erect chisel-like portions separated by a very narrow shallow slit. Tenth tergite completely divided into two lateral rounded lobes, bearing posteriorly the heavy basal portion of the supra-anal apparatus, to which is attached the rather stout curved supra-anal process. The tip of the process is bent slightly backward, and there is a very small subterminal bump anteriorly (Fig. 14). Ninth sternite with a thick, ovoid basal process, whose lower surface is covered with brown spinules. Subanal lobes joined by a membrane along part of their length, but separate for the terminal third; the terminal process of the fusion plate very slender, not much exceeding the lobes in length.

Female: Eighth sternite with a posterior median unsclerotized region about 1/5 of the length of the sternite; the remainder fairly evenly sclerotized, or with a variable density of sclerotization, one form of which is shown in Fig. 16.

Holotype ♂, allotype ♀, 4 dwarf and 3 long-winged ♂ paratypes: Hyalite Creek, Township 3 South, Range 5 East, Section 23, Gallatin Co., Montana, May 8, 1952, R. Hays. Holotype and allotype are in the INHS collection.

Other paratypes from same location as holotype: May 10, 1951, 1 dwarf ♂; May 9, 1952, 3 dwarf and 2 long-winged ♂, 1 ♀; May 11, 1952, 1 ♀. Paratypes have been placed in the SGJ and MSC collections.

Other material: Hell Roaring Creek at Highway No. 191, Gallatin Co., Montana, R. Hays and W. Alvord, May 13, 1952, 1 ♀.

This is one of several new species of stoneflies turned up by Mr. R. Hays' tireless collecting in southern Montana. The male found in 1951 served as a stimulus for the intensive effort which produced a good series of specimens in 1952. The span of emergence is evidently narrowly limited, since collections earlier and later in the season contained no *hyalita*. The type locality, Hyalite Creek, contains a rich and varied fauna of stoneflies, including such rarities as *Utaperla sopladora* and *Alloperla albertensis*.

Isocapnia integra Hanson 1943

1943. *Isocapnia integra* Hanson, Bull. Brooklyn Entomol. Soc. **38**, 160, Fig. 6. The holotype is Banks' female cotype of *grandis*.
1907. *Arsapnia grandis* Banks, in part, ♀ cotype only (the ♂ cotype was designated as lectotype of *grandis* by Frison, 1942).

Length of body, dwarf ♂ 7 mm, long-winged ♂ 7.5 mm, ♀ 9–10 mm; to tip of wings, long-winged ♂ 9 mm, ♀ 12–14 mm. Costal cross veins two to four before the tip of *Sc*, one to two beyond. Color of ♂ grey brown, of ♀ lighter brown, in dry specimens.

Male (not previously described): Abdominal segments and genitalia very similar in general to *fraseri*; the supra-anal process thicker in its lower half (Fig. 9).

Female: Eighth sternite usually light yellow in color, except at the sides (but in a few specimens it is dark except along the hind margin); its hind margin not recessed, straight or slightly rounded, fully sclerotized (Fig. 12).

Nymph: A number of ♀ exuviae were taken; they have long profuse body hairs, and are indistinguishable from *fraseri*.

Isocapnia integra may be compared with the closely related *fraseri* as follows:

	integra	*fraseri*
Color of body and wings	Brownish	Grey to black
♀ subgenital plate	Flush with the margin of the sides of the segment, or even slightly produced	Varies from almost flush to considerably recessed
Lobe on 9th sternite of ♂	Of moderate size in the two specimens at hand	Varies from small to moderate size
Process of 9th tergite	Slightly broader, flatter, and not as deeply notched as in *fraseri*	—
Habitat	Clear mountain streams and lakes	Large muddy river near sea level

Material examined is as follows:

ALBERTA

Banff. June 17, 1901, 1 ♀ (holotype—MCZ).
Banff. J. C. Bradley. June 22, 1908, 1 ♀ (CUC).
Banff. C. B. D. Garrett. June 11, 1922, 2 ♀ (CNC).
Spray River, Banff. W. E. Ricker. June 5, 1957, 1 dwarf ♂, 3 ♀, 6 exuviae; May 28, 1958, 1 long-winged male, 1 ♀, 5 exuviae.
Consolation Lake, Banff National Park. W. E. Ricker. June 1, 1958, 1 ♀, 1 exuvia (previous year's hatch).
A cirque lake tributary to Wolverine Creek, near Mts. Brett and Bourgero, Banff National Park. Karl Ricker. June 28, 1958, 10 ♀.

The 1957 and 1958 specimens of *integra* from the Spray River were taken from cobwebs on the water-gauging station about half a mile above the mouth. It was not clear whether these had emerged earlier the same season or during the previous season. The "live" captures at Banff earlier in the century suggest emergence throughout June, but the upper Spray River has recently been dammed and regulated, so its temperature regime is doubtless different from formerly.

Isocapnia japonica Kohno 1953

1953. *Isocapnia japonica* Kohno, Philippine J. Sci. **82**(2), 203. Male, female.

This species has both dwarf and normal males, the supra-anal process being slender, but distinctly bent backward in its outer third. The ♀ subgenital plate is recessed but apparently entire. Costal cross veins are three before the end of *Sc*, none beyond it, in the forewing and the hind wing figured by Kohno. These characters relate the species to the *hyalita* group; absence of the terminal cross vein is rare but not unknown in small specimens of *fraseri*, and is a natural development for the extremely small *japonica* (♀ 7.5 mm body length).

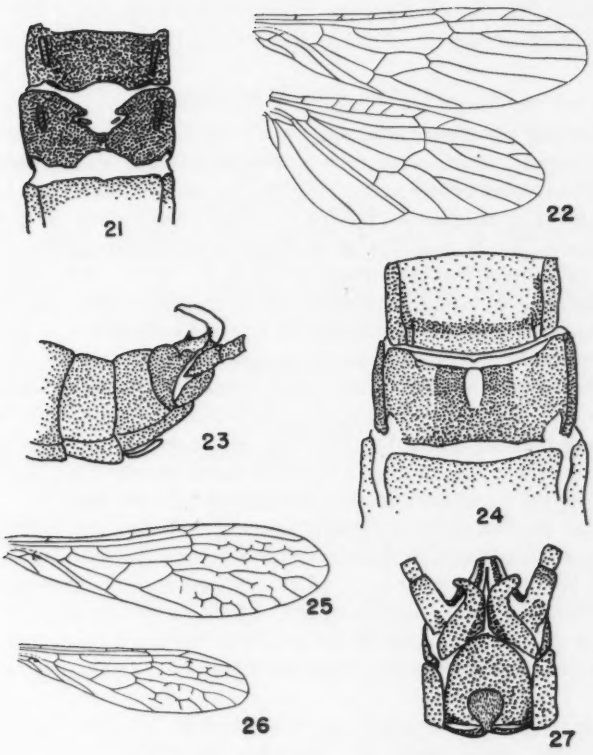
Isocapnia ***kudia*** new species

Length of body of ♀ 12 mm, to tip of wings, 17 mm. Body dark, wings rather heavily infuscated with brown (about as in *I. grandis*). Costal cross veins of the forewings four to six before the end of *Sc*, one or two beyond it (Fig. 22). The thoracic sterna are typical of *Isocapnia*.

Female: Eighth sternite non-sclerotized medially except for a narrow transverse bridge; sides of the posterior membranous area irregular and containing a pair of small sclerotized "islands" (Fig. 21), that of the left side the better developed.

Holotype ♀: Kudia River, Amagu, Siberia, USSR, July 1923, Cockerell (in USNM).

This large species extends the known range of the genus onto the mainland of Asia. Dr. B. D. Burks of the USNM very kindly looked up the record of Dr. Cockerell's trip to Siberia, and found that "Amagu" is the small town shown on modern maps as "Amgu", lying on the coast of the Maritime Province 330 air miles north-northeast of Vladivostok, in 137° 40′ East longitude and 45° 50′ North latitude.



Isocapnia ***missourii*** new species

Length of body, dwarf ♂ 9.5 mm, ♀ 12–13 mm; to tips of wings, ♀ 13.5–15 mm. Three to five cross veins before end of *Sc*, one beyond it.

Very close to *fraseri*, differing only in its much larger size and relatively somewhat longer and more slender supra-anal process (Fig. 13). The 8th sternite of the Gallatin River ♀ has the hind margin recessed (Fig. 15), the central part of the recessed portion being sclerotized (though light in color) right to the hind margin, while to its side is a shallow unsclerotized area. However, this latter probably results from the teneral condition of the specimen; one of the Utah females has the plate sclerotized solidly across.

Holotype ♂: Missouri River, Toston, Montana. R. Hays. April 4, 1952.

Other material has been examined as follows:

MONTANA

West Gallatin River at Squaw Creek bridge, Gallatin Co. R. Hays and W. Alvord. May 6, 1951, 1 ♀.

UTAH

Brigham Canyon. F. C. Harmston. May 1, 1937, 1 ♀; Knowlton and Bischoff, April 18, 1939, 1 ♀.

I hesitate to describe an allotype, while lacking a specimen from the same river as the holotype, but there seems little doubt of the association given.

Isocapnia ***mogila*** new species

Length of body, ♂ 6 mm, ♀ 8 mm; to tips of wings, ♂ 9 mm, ♀ 11.5 mm. Wings moderately tinted with brown, the veins dark brown; three to four subcostal cross veins before end of *Sc*, one beyond. The forewing venation of all specimens is irregular in having numerous incomplete cross veins from the branches of *Rs*, *M*, and Cu_1 (Figs. 25, 26); similar irregularity is slightly developed in some of the hind wings.

Male: Tergites 1–4 with a median non-sclerotized stripe narrowing posteriorly; tergites 5–10 fully sclerotized; tergites 6–8 with the front margin sinuous, increasingly so on the more posterior of these segments. Sternites heavily sclerotized, the hind fourth of 8 lighter centrally; sternite 9 with a broad lobe and suspensory band (Fig. 27). Subanal lobes fully sclerotized, rounded, and curved outward at the tips, which exceed the structures dorsal to them (Fig. 27). Fusion plate produced into a slender tip. Basal segment of the cercus excavated and heavily sclerotized near the base.

Supra-anal process short, recurved forward, with a slight hook at the tip, attached to a broad basal plate which has a short spur centrally (Fig. 23).

Female: Eighth sternite (Fig. 24) slightly recessed, its hind margin bordered by a narrow transverse unsclerotized rolled marginal fold; behind this, centrally, is posterior unsclerotized spot, this bordered by broad sclerotized bands, the latter somewhat set off on each side by a triangular unsclerotized patch.

Holotype ♂ and 1 ♂ paratype: Cow Creek, Douglas Co., Oregon, at highway No. 99. S. G. Jewett, Jr. November 22, 1955.

Allotype ♀: Grave Creek, tributary of the Rogue River, Oregon. S. G. Jewett, Jr. March 1, 1950. Holotype and allotype are deposited in the collection of the California Academy of Sciences, San Francisco.

Mr. Jewett has asked me to describe this unusual species, evidently a rarity in the Oregon fauna.

Isocapnia spenceri Ricker 1943

1943. *Isocapnia spenceri* Ricker, Indiana Univ. Publs. Sci. Ser. No. 12, 91, Figs. 67–70. Long-winged male, female.
1943. *Isocapnia spenceri*, variety *thujae* Ricker, Indiana Univ. Publs. Sci. Ser. No. 12, 92, Fig. 73. Dwarf male. New synonymy.

Nymph: Exuviae collected with adults at Haslam and Cameron Creeks on Vancouver Island are very hairy and, when dry, have the characteristic out-turned tips of the cerci. Figure 18 shows the outline of the supra-anal sheath of a dwarf male; in a long-winged male it would probably be a little larger relative to the body size.

The repeated occurrence of the short-winged dwarf *thujae* type with long-winged *spenceri* makes it clear that they belong to the same populations; also,

the structural differences described in 1943 are not consistently present, though all features of the genitalia tend to be more lightly developed in the dwarf form.

The specimens described in 1943, only four in all, were from the Chilliwack River at Vedder Crossing, B.C. More abundant material is now available, of which I have examined the following:

BRITISH COLUMBIA

Cameron Creek above Cameron Lake, Vancouver Island. W. E. Ricker, May 9, 1954, 1 dwarf ♂, 1 exuvia; April 8, 1955, 2 dwarf ♂, 2 exuviae; April 15, 1956, 1 dwarf ♂, 2 ♀.
Capilano River, West Vancouver. W. E. Ricker. April 18, 1957, 10 dwarf ♂, 2 ♀.
Haslam Creek at Cassidy, south of Nanaimo, Vancouver Island. W. E. Ricker. March 16, 1952, 1 dwarf ♂, 1 long-winged ♂, 7 ♀, 3 exuviae; March 19, 1952, 6 dwarf ♂, 2 long-winged ♂, 9 ♀, 11 exuviae; March 26, 1952, 2 dwarf ♂, 1 long-winged ♂; April 5, 1952, 1 dwarf ♂.
Lakelse River near Terrace. F. Neave. June 15, 1950, 1 ♀.
Seymour Creek, North Vancouver. W. E. Ricker. April 18, 1957, 1 ♀.

CALIFORNIA

Trinity River, 7 miles south of Trinity Center. G. Black. March 2, 1955, 1 dwarf ♂ (SGJ).

OREGON

Corvallis. Don Coons. March 25, 1938, ♀ (INHS).
Hillsboro. K. Gray and J. Schuh. March 19, 1936, ♀ (INHS).
Santiam River near Jefferson, Marion Co. S. G. Jewett, Jr. March 4, 1948, 2 long-winged ♂, 8 ♀ (SGJ).
Willamette River at Harrisburg. S. G. Jewett, Jr. March 4, 1948, 2 dwarf ♂ (SGJ).

EMBRYOLOGY OF PIMPLA TURIONELLAE (L.) (HYMENOPTERA : ICHNEUMONIDAE)[1]

JOAN F. BRONSKILL

Abstract

The embryogenesis of the parasitic ichneumonid *Pimpla turionellae* (L.) is more similar to that of the aculeate *Apis mellifera* L. and the chalcidoid *Eurytoma aciculata* Ratz. than to that of other Hymenoptera that have been studied, including the ichneumonid *Banchus femoralis* (Thoms.). The fact that *P. turionellae* is a parasitoid has had little influence on its embryogenesis, though in some other parasitic insects embryonic development has been simplified and the embryo hatches before histogenesis of its larval tissues is completed.

Introduction

Pimpla turionellae (L.) is an arrhenotokous monembryonic ichneumonid parasitoid of the European pine shoot moth, *Rhyacionia buoliana* (Schiff.) (62), and of several other Lepidoptera. Though other parasitic Hymenoptera have been the subject of extensive embryonal research, ichneumonids have received little attention. Kulagin (38) worked on *Mesochorus splendidulus* Grav. and Rietra (49) on *Exidechthis canescens* (Grav.); Crawford (13) made some embryological notations in his biological study of *Glypta rufiscutellaris* Cress.; and Bledowski and Krainska (5) did a noteworthy embryonic study on *Banchus femoralis* (Thoms.). However, these studies on ichneumonids did not include detailed descriptions of the external anatomical changes that occur during embryogenesis, or of the early differentiation of the germ cells, their migration during embryonic development, and their eventual formation into the larval gonad. In this paper external and internal anatomical changes that occur in the egg of *P. turionellae* from the time of oviposition until larval hatching are described and compared with similar phases of development in several other species of Hymenoptera.

Materials and Methods

P. turionellae can be reared easily throughout the year on the wax moth, *Galleria mellonella* (L.). The parasitoid adults feed on whole raisins, squashed host pupae, and 10% aqueous honey solution applied to small pads of absorbent cotton. The females readily attack host pupae after a preoviposition period of approximately 8 days. Host pupae were removed from their cocoons and placed in a cage with *P. turionellae*, on corrugated cardboard strips (2 in. × 3 in.). As the host pupae lay securely in the depressions of the corrugations, the *P. turionellae* females readily oviposited on them. The corrugated cardboard aided in holding the pupae rigid; otherwise the females

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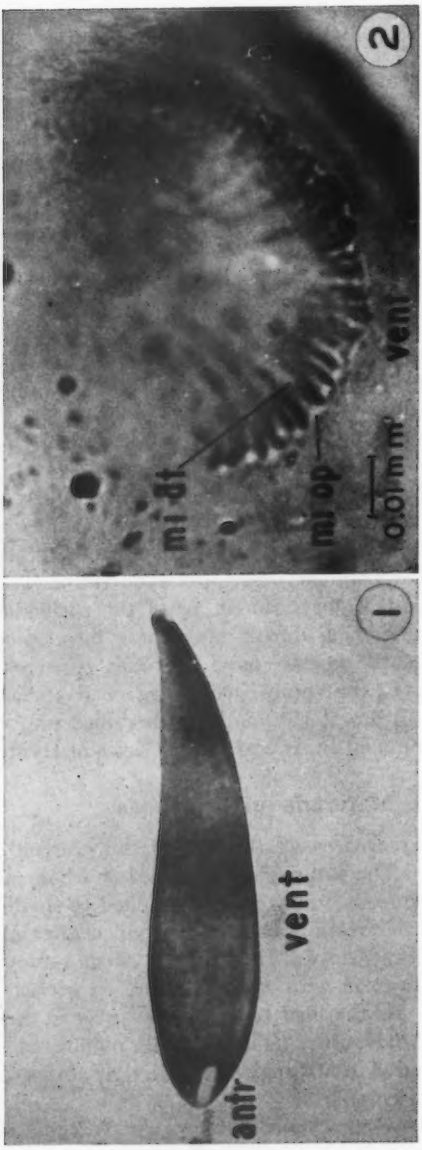


FIG. 1. A living *Pimpla turionellae* egg dissected from a *Galleria mellonella* pupa immediately after deposition, showing its cephalocaudal and dorsoventral orientation. ×47
FIG. 2. The anterior tip of a *P. turionellae* chorion showing the micropylar openings and ducts.

had great difficulty in piercing the heavily sclerotized pupal cases. Immediately after the pupae were parasitized, they were removed from the cages and placed individually in 1½-in. shell vials plugged with absorbent cotton. The time of oviposition was recorded on each vial. The parasitized host pupae were incubated at 23±0.5° C and a relative humidity of 76±1%. Under these conditions *P. turionellae* completes its embryonic development and hatches in 30±1 hours.

The parasitized host pupae were dissected in Ringer's (40, p. 77) solution at 15-minute intervals during the first 2 hours after deposition, and at hourly intervals for the remainder of the embryonic period. Carnoy's (23, p. 44, #89) fixative and alcoholic Bouin's (23, p. 325, #754) fixative were used to preserve the embryos. After 5 minutes of fixation, each embryo was pricked and the chorion removed. The embryos were then placed in fresh fixative for 5±1 hours. Ethanol (80%) was used to wash the fixatives from the embryos. All embryos were stored in ethanol (70%).

The chorion and the serosa were removed from whole fixed embryos to observe the external anatomical changes that occur during embryonic development.

Staining with Grenacher's (23, p. 134, #265) borax carmine enhanced clarity of anatomical detail in these embryos. As the chorion of *P. turionellae* eggs is thin, colorless, and transparent, the external changes that occur during embryonic development were readily observed in living embryos kept in Ringer's solution.

Fixed embryos were prepared for sectioning by dehydrating in ethanol and clearing in benzol before impregnation with Tissuemat [3] (melting point 56–58° C). All embryos were sectioned at 4 microns. Mallory's (40, p. 62) phosphotungstic acid haematoxylin and Delafield's (40, p. 57) haematoxylin with eosin counterstain were used to stain the sectioned material. Illustrations of the whole and sectioned embryos were prepared with the aid of a camera lucida and a microprojection prism. The terminology of Snodgrass (55, 57) and Beirne (4) was followed in the preparation of Fig. 11.

Structure of the Egg

The egg (Fig. 1) is elongate, obtusely pointed cephalically, and acutely pointed caudally, with its greatest width one-third the distance from the anterior end. It is bilaterally symmetrical, slightly convex ventrally, and somewhat concave dorsally (Fig. 1), and is light ivory in color when newly laid. Its orientation is similar to that of the ichneumonid *Banchus femoralis* (5) and other Hymenoptera, including the mason bee, *Chalicodoma muraria* (F.) (11), the honeybee, *Apis mellifera* L. (42), and the chalcidoids *Trichogramma evanescens* Westw. (21) and *Mestocharis militaris* R.-Kors. (30). The egg of the ichneumonid *Glypta rufiscutellaris* (13) and of the sphecid *Ammophila campestris* Jur. (3) has the caudal end larger and more obtusely pointed than the cephalic end. Though Silvestri (54) stated that the egg of

[3] Fisher Scientific Co., Toronto 8, Ontario.

P. turionellae is 1.32 mm long and 0.22 mm wide, newly laid eggs used in the present study were 1.615 ± 0.11 mm long and 0.265 ± 0.017 mm wide at their widest point.

The mature egg follows constantly Hallez's (24) law of orientation within the polytrophic ovariole according to its cephalocaudal axis only; that is, the anterior pole of the egg is directed cephalad within the abdomen of the female, and the posterior pole lies caudad. The positions of the dorsal, ventral, and lateral sides vary, and do not agree with those of the mother in every case. This is especially true when all the ovarioles contain mature eggs and are crowded. The embryo retains the original cephalocaudal axis during its entire development, and there is little blastokinesis. However, the embryo rotates 180° on its longitudinal axis immediately before eclosion.

The chorion is transparent, delicate, and flexible. Though it appears smooth, it has indistinct follicular cell markings on its external surface; these become visible only after the chorion is removed from the egg and allowed to dry. The darkly pigmented micropylar ducts lie in a semicircle at the anterior tip of the chorion (Fig. 2). The external openings are ventral whereas the ducts are directed centrad. During embryonic development, the chorion and vitelline membrane at the posterior end of the egg collapse, after the embryonic constituents withdraw from this region. The embryonic materials also withdraw slightly from the anterior pole. Crawford (13) observed a similar withdrawal of the embryonic material from the cephalic and caudal poles in the egg of *Glypta rufiscutellaris*. Though the egg increases in size soon after deposition in the ichneumonid *G. rufiscutellaris* (13), and the chalcidoids *Prestwichia aquatica* Lubb. (29) and *Caraphractus reductus* R.-Kors. (31), this does not occur in *P. turionellae*.

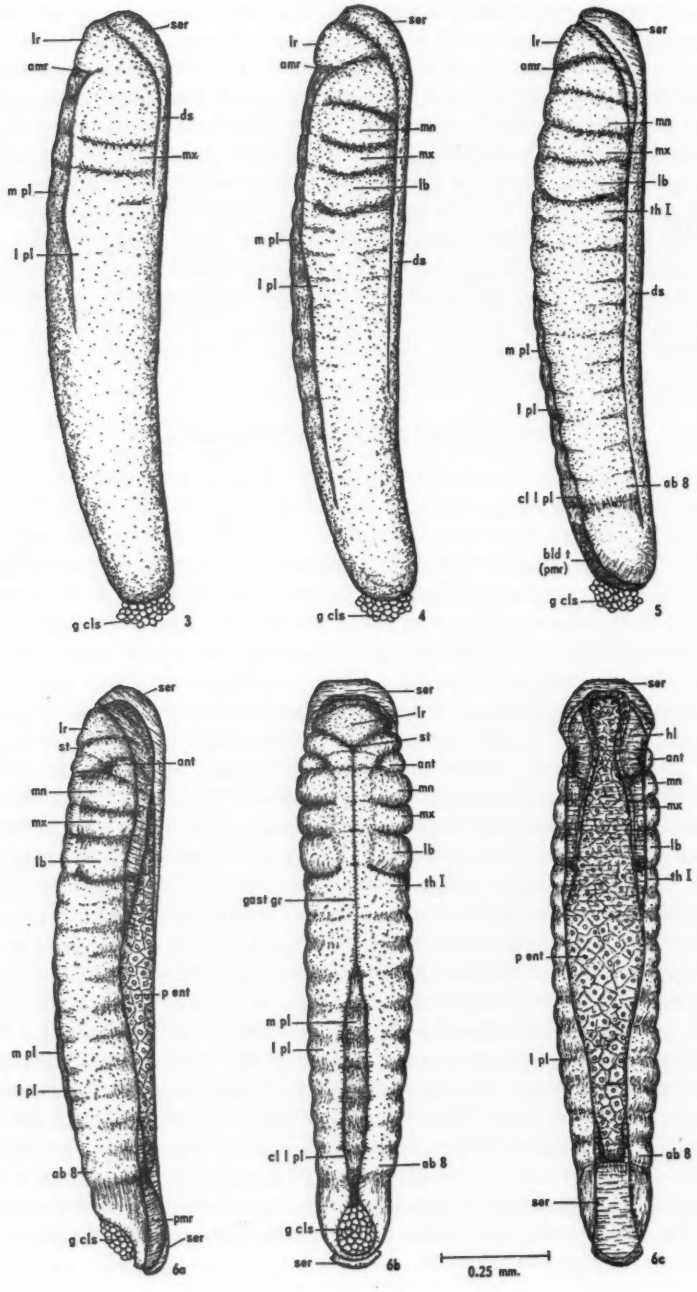
The thin, noncellular vitelline membrane is closely associated with the inner wall of the chorion. The membrane retains its association with the chorion after the embryonic tissues withdraw from the anterior and posterior ends of the egg. A thin layer of peripheral protoplasm (periplasm) is present in the newly laid ovum (Fig. 38, *pplm*) and a plasma membrane is visible at its periphery. The periplasm is continuous with the internal protoplasmic reticulum (Fig. 38, *ret*). The main deutoplasmic substances in the insect ovum are the protein yolk bodies, the lipide yolk bodies, and glycogen particles (7). In sectioned eggs of *P. turionellae* stained with Mallory's phosphotungstic acid haematoxylin, small, irregularly shaped deutoplasmic granules that stain pink and purple fill the spaces between the mauve protoplasmic reticulum (Fig. 38, *dplm*, *ret*). The deutoplasm is eosinophilic and the protoplasm is basophilic in sections stained with Delafield's haematoxylin and eosin. Though yolk is common in monembryonic Hymenoptera it is lacking in the chalcidoids *P. aquatica* (29) and *C. reductus* (31).

Neither maturation of the egg nucleus nor fusion of the male and female pronuclei was observed. Maturation occurs before deposition in the ichneumonid *Banchus femoralis* (5).

The germinal cytoplasm (oösome) lies near the caudal pole in the central yolk mass of the newly laid *P. turionellae* egg. In preparations stained with Mallory's phosphotungstic acid haematoxylin (Fig. 38, *osm*), it appears as an extremely vacuolated body that stains either dark purple or pinkish yellow, depending on the fixative used. The oösome is basophilic in sections stained with Delafield's haematoxylin. It is generally oval in shape, approximately 42 microns long and 25 microns wide, but in some cases appears almost spherical. Within the zone of growth of the ovarioles, the oösome is readily observed differentiating at the posterior end of the oöcytes. Silvestri (53), Hegner (25), Gatenby (21, 22), and Ivanova-Kazas (29, 31, 35) observed a similar basophilic structure at the posterior pole in the ova of several monembryonic parasitic Hymenoptera; its granules later became dispersed in the cytoplasm of the cleavage cells that entered this region, differentiating them into germ cells.

Cleavage and Blastoderm Formation

Superficial cleavage begins between 1 and 2 hours after the egg of *P. turionellae* is laid. Cleavage nuclei were first observed at the site of the presumptive gnathal region (cleavage center). Their migration to the periphery begins at the cleavage center. Each cleavage nucleus is surrounded by a stellar mass of protoplasm. As the cleavage nuclei migrate to the periphery their numbers increase by further mitotic division. The migrating cleavage nuclei appear 'comet-shaped' as the protoplasm associated with each projecting centrad forms a 'tail' (Fig. 12, *cn*). At this time the cleavage nuclei are indistinct during interphase as their nuclear material has little affinity for stains. Before the cleavage nuclei enter the periplasm, the latter thickens slightly on the dorsal, ventral, and lateral surfaces of the egg. The cleavage nuclei slowest in reaching the periphery are those that go to the caudal pole. All the cleavage nuclei reached the periphery within 4 hours after deposition to form the blastema (Fig. 13, *blt*), and lay against the vitelline membrane, giving the surface of the egg a rough appearance. The blastema (43) is the outer nucleated layer of periplasm formed by the entrance of the cleavage nuclei within this periplasm. It is a syncytium and should be distinguished from the blastoderm, which is formed only after the cell walls have developed. In *P. turionellae* the protoplasm (the 'tails') of the cleavage nuclei becomes associated with the blastema to form the inner periplasm (Figs. 13, 14, *i pplm*), which later becomes the proximal cytoplasm of the blastodermal cells. After further mitotic division, the blastemal nuclei move inward to lie centrally in the periplasm (Fig. 14). A similar movement of the blastemal nuclei in the periplasm has been observed in the sphecid *Ammophila campestris* (3). At the same time, cell boundaries begin to form, at first as furrows in the external surface that slowly extend into the periplasm as actual membranes (Fig. 14). Cell walls are visible in the central and anterior regions of the egg before they appear caudally.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The blastodermal cells are generally complete in the 6-hour egg and appear as columnar cells with slightly oval nuclei and highly basophilic cytoplasm (Fig. 15, *bld*). They are of a similar columnar nature in the ichneumonid *Glypta rufiscutellaris* (13). Small vacuoles occur in the cytoplasm of many cells on the proximal side of the blastodermal nuclei (Fig. 15, *vac*). They appear to be the remnant of the demarcation between the blastema and the inner periplasm, and soon disappear. Vacuoles of a similar nature were noted in the blastoderm of the fly *Phaenicia sericata* (Mg.) (18). The blastoderm in *Glypta rufiscutellaris* (13), *Ammophila campestris* (3), and *P. turionellae* is of uniform thickness throughout; however, it is thicker ventrally than dorsally in *Apis mellifera* (42, 51) and in the sawfly *Nematus* (*Pteronidea*) *ribesii* (Scop.) (52).

The cleavage nuclei remaining within the deutoplasm become the primary yolk cells (vitellophags) (Fig. 15, *yc*). During differentiation of the cell membranes in the blastoderm, these yolk cells become concentrated centrally throughout the length of the yolk mass. They lie in clumps surrounded by the protoplasm that remains in the yolk. Secondary yolk cells do not appear to be formed in *P. turionellae*, if one considers secondary yolk cells as those cells that return into the yolk after the blastoderm forms, as defined by Johannsen and Butt (37). According to Rabinowitz (48), in *Drosophila melanogaster* Mg. tertiary yolk cells (nuclei) are formed from the pole cells that pass into the yolk by interblastodermal migration. Recently, Poulson (45, 46) showed that these yolk cells (of pole cell origin) become part of the definitive mid-gut epithelium. Though some pole cells (germ cells) appear to migrate similarly to form tertiary yolk cells in *P. turionellae*, there is not enough evidence at this time to reach a definite conclusion.

Formation of Germ Cells

During early cleavage the oösome migrates caudad and lies at the posterior tip of the egg. In 2-hour eggs it presses against the periphery, causing a slight bulge in the ventral surface of the egg near the caudal end. Soon the definite oval form of the oösome is lost as its germinal cytoplasm becomes dispersed throughout the caudal pole before the cleavage nuclei enter. Only a thin layer of periplasm is now present at the posterior end, whereas the periplasm has thickened somewhat, as noted previously, along the dorsal, ventral, and lateral surfaces before the cleavage nuclei entered. Several cleavage

FIGS. 3–6. *P. turionellae.* Illustrations prepared from living embryos and stained whole mounts as viewed under reflected light. Chorion and vitelline membrane not shown. 3. Lateral view of a 7-hour embryo, showing the initial differentiation of the anterior mid-gut rudiment (*amr*), labrum (*lr*), anterior serosal cap (*ser*), dorsal strip (*ds*), maxillary segment (*mx*), and middle and lateral plates (*m pl, l pl*). The germ cells (*g cls*) project from the caudal pole. 4. Lateral view of an 8-hour embryo showing further differentiation of the gnathal lobes (*mn, mx, lb*), the dorsal strip (*ds*), and the middle and lateral plates (*m pl, l pl*). 5. Lateral view of a 9-hour embryo, showing the segmentation of the abdominal region and the extension of the clefts of the lateral plates (*cl l pl*) around the caudal pole. The germ cells (*g cls*) still project caudad. 6. Lateral (*a*), ventral (*b*), and dorsal (*c*) views of a 10-hour embryo, showing severance of the anterior serosal cap (*ser*) from the rudimentary amnion, the ventromesial union of the lateral plates (*l pl*), the ventrocephalic migration of the germ cells (*g cls*), and the differentiation of the posterior serosal cap (*ser*).

For abbreviations used in the figures see p. 688.

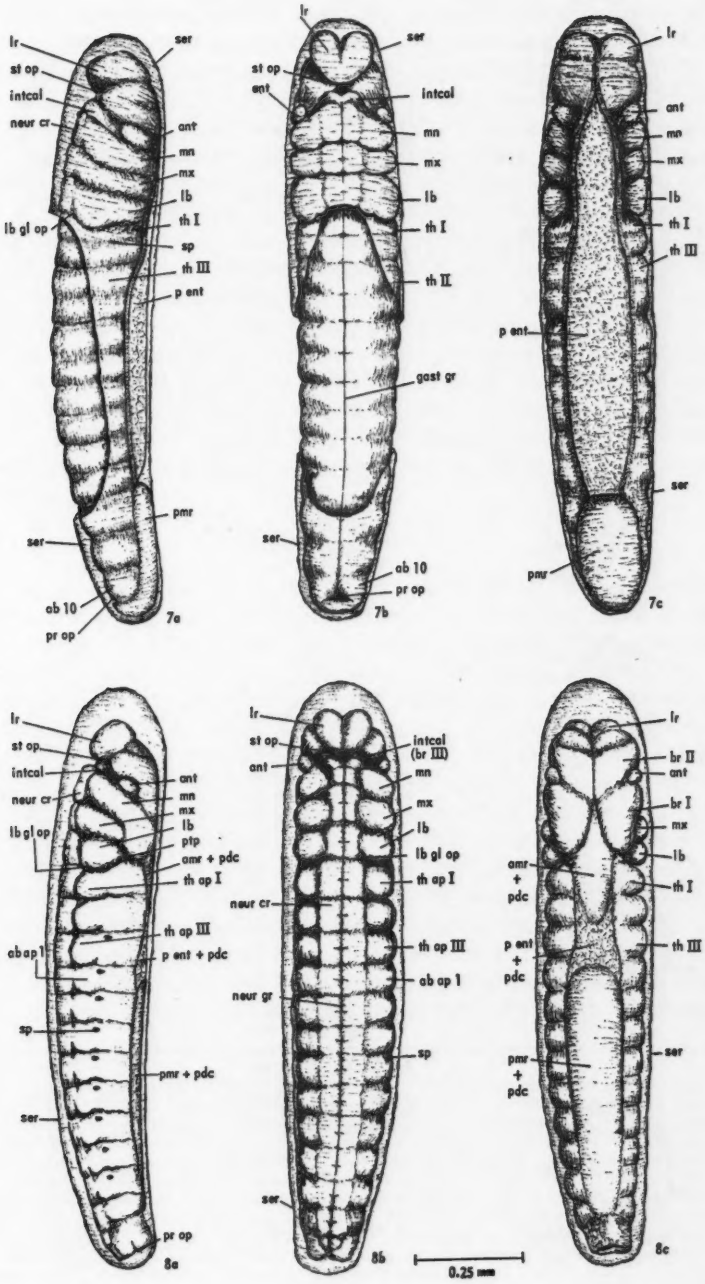
nuclei enter the posterior germinal cytoplasmic region and become differentiated as germ cells. The initial germ cells undergo synchronous mitotic division at this time, forming the germ cell mass at the caudal pole of the egg (Fig. 3, *g cls*). Thus, the germ cells of *P. turionellae* are polynuclear in origin, as in some other insects, including *Aphidencyrtus aphidivorus* (Mayr), *Trichogramma semblidis* (Auriv.) (53), and *Drosophila melanogaster* (26). The blastoderm becomes completed caudally and separates the germ cells from the rest of the embryonic material (Fig. 39). As the nuclei of the germ cells are somewhat larger than those of the somatic cells and as their cytoplasm is less basophilic, their migration and development into the larval gonad are readily discernible throughout the remainder of the embryonic development. Ivanova-Kazas (29–31, 35) observed the early differentiation of the germ cells at the caudal pole in the chalcidoids *Prestwichia aquatica*, *Mestocharis militaris*, *Caraphractus reductus*, and *Eurytoma aciculata* Ratz.

Formation of Germ Band, Dorsal Strip, and Inner Layer

Several events leading to the formation of the embryo begin almost simultaneously at 7 hours. The blastoderm differentiates ventrally and laterally throughout its length into the germ band, and dorsally into the extraembryonic blastoderm (anterior serosal cap and dorsal strip) (Fig. 3). These developments are clearly visible in living embryos. A cleft appears at the anterior pole that demarcates the anterior serosal cap (*ser*) from the ventral region of the germ band. This cleft continues caudad as a slight ridge on each side of the mid-dorsal line. These demarcate the dorsal strip (*ds*) from the lateral plates (*l pl*) of the germ band. By the ninth hour the dorsal strip has differentiated to the caudal pole (Fig. 5, *ds*).

The ventral blastodermal cells thicken, and the anterior mid-gut rudiment differentiates mid-ventrally near the anterior end of the germ band (Figs. 3, 18, *amr*). At the same time a narrow cleft appears ventrolaterally on either side of the germ band just caudad of the anterior mid-gut rudiment. This cleft progresses quickly caudad to demarcate the middle (mesodermal) and the lateral (ectodermal) plates of the germ band (Figs. 3, 4). Extensive mitotic activity in the middle plate coincides with the appearance of the clefts, and this results in a proliferation of the middle plate cells (Fig. 19, *m pl*). Farther caudad, mitosis is just beginning in the middle plate, and the spindles appear perpendicular to the surface of the embryo (Fig. 20). Just beyond this region, there is only a slight indication of the ventrolateral clefts, and the middle plate is not, as yet, mitotically active but still consists of a single layer of columnar cells (Fig. 21).

FIGS. 7–8. *P. turionellae*. Illustrations prepared from living embryos and stained whole mounts as viewed under reflected light. Chorion and vitelline membrane not shown. 7. Lateral (*a*), ventral (*b*), and dorsal (*c*) views of an 11-hour embryo, showing further development of the head region and of the serosa, the appearance of the labial gland (*lb gl op*) and tracheal (*sp*) invaginations, the cephalic proliferation of the posterior mid-gut rudiment (*pmr*), and the complete submergence of the germ cells within the embryo. 8. Lateral (*a*), ventral (*b*), and dorsal (*c*) views of a 15-hour embryo completely enveloped within the serosa, showing the provisional dorsal closure (*pdc*), the neural crests (*neur cr*), the thoracic and the abdominal appendages (*th ap, ab ap*), and the proximity of the anterior and posterior mid-gut ribbons (*amr, pmr*).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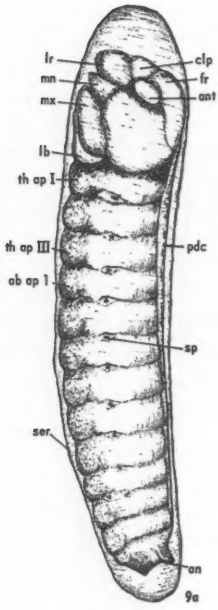

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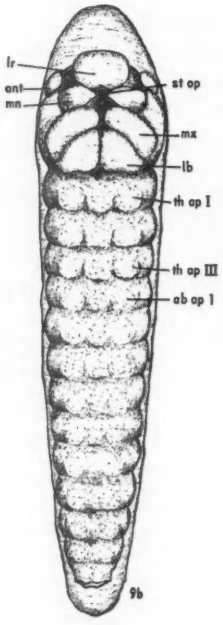

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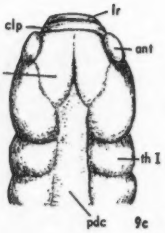

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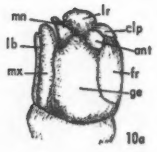

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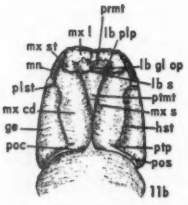

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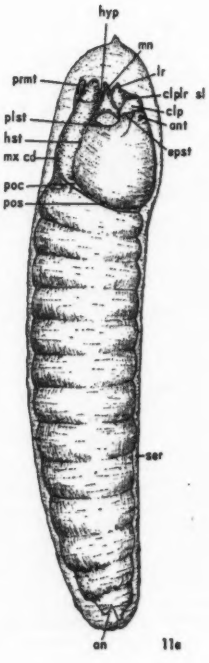

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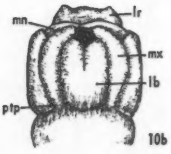

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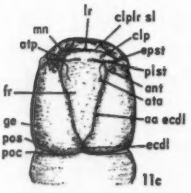

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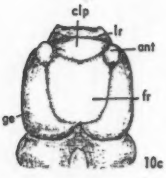

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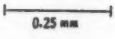


The inner layer forms from a sinking-in of the thickened middle plate (Fig. 19, *m pl*) and its overgrowth by the two lateral plates (*l pl*). This process begins just behind the anterior mid-gut rudiment and continues to the posterior end of the germ band. Figure 23 illustrates the extent of the inner layer (mesoderm) in a 9-hour embryo. By 11 hours the middle plate has sunk completely beneath the lateral plates, which have united mid-ventrally to form the ectoderm (Fig. 7*b*). Gastrulation in *P. turionellae* is similar to that in the sphecid *Ammophila campestris* (3) and the chalcidoid *Eurytoma aciculata* (35), in that the middle plate thickens before it sinks beneath the lateral plates. As the middle plate of *Apis mellifera* sinks below the lateral plates, mitotic activity occurs (42). In the ichneumonid *Banchus femoralis* the inner layer develops by tangential division of the blastodermal cells and ventral accumulation of excess cleavage cells beneath the blastoderm (5). In the chalcidoid *Prestwichia aquatica* (29) and the sawfly *Nematus ribesii* (52) the inner layer forms by a proliferation and dispersal of cells from the mid-ventral region of the germ band. Thus, gastrulation occurs in diverse ways among the Hymenoptera and even differs in different species of the same family.

Segmentation

According to Bodenstein (6, p. 781), "The first segments to be marked off in the germ band are those of the second maxillary and first thoracic segments"; however, in *P. turionellae* the first maxillary segment is demarcated by segmental grooves (Fig. 3, *mx*) immediately after the middle and lateral plates differentiate. Segmentation of the middle and lateral plates progresses anteriorly and posteriorly from this point and occurs simultaneously with the cephalocaudal differentiation and mesial migration of the lateral plates (Figs. 3–7). The middle plate segments slightly in advance of the lateral plates. Segmentation also occurs early in *Apis mellifera* (42, 51). The ichneumonid *Banchus femoralis*, on the other hand, does not become segmented until late in its embryonic development; muscles and the labial glands are differentiated in this insect before segmentation (5). Though segmentation in *P. turionellae* appears to be complete by 11 hours (Fig. 7), the somites are not so distinct at this time as they are in the mature embryo (Fig. 11*a*). The first instar larva has a sclerotized head capsule and 13 body segments (3 thoracic and 10 abdominal segments). The 10th abdominal

FIGS. 9–11. *P. turionellae*. Illustrations prepared from living embryos and stained whole mounts as viewed under reflected light. Chorion and vitelline membrane not shown. 9*a*. Lateral view of an embryo at 22 hours, showing the cephalic migration of the gnathal lobes (*mn*, *mx*, *lb*). 9*b*. Ventral view of an embryo at 22 hours. Neural crests are no longer visible. 9*c*. Dorsal view of the head at 22 hours. Provisional dorsal closure (*pdc*) appears as a distinct tissue. Serosa not shown. 9*d*. Dorsal view of the posterior end of an embryo at 22 hours. Serosa not shown. 10. Lateral (*a*), ventral (*b*), and dorsal (*c*) views of the head at 24 hours. The labial lobes (*lb*) have fused mid-ventrally. Serosa not shown. 11*a*. Lateral view of embryo at 29 hours. The head is now sclerotized. 11*b*. Ventral view of the head at 29 hours. Serosa not shown. 11*c*. Dorsal view of the head at 29 hours. Serosa not shown.

segment bears a pair of dorsal and a pair of ventral terminal lobes. Segmentation of the dorsal region of the embryo coincides with the mesiodorsal movement of the mesodermal tissue. With the formation of the dorsal blood vessel mesiodorsad, segmentation of the epidermis becomes completed above it.

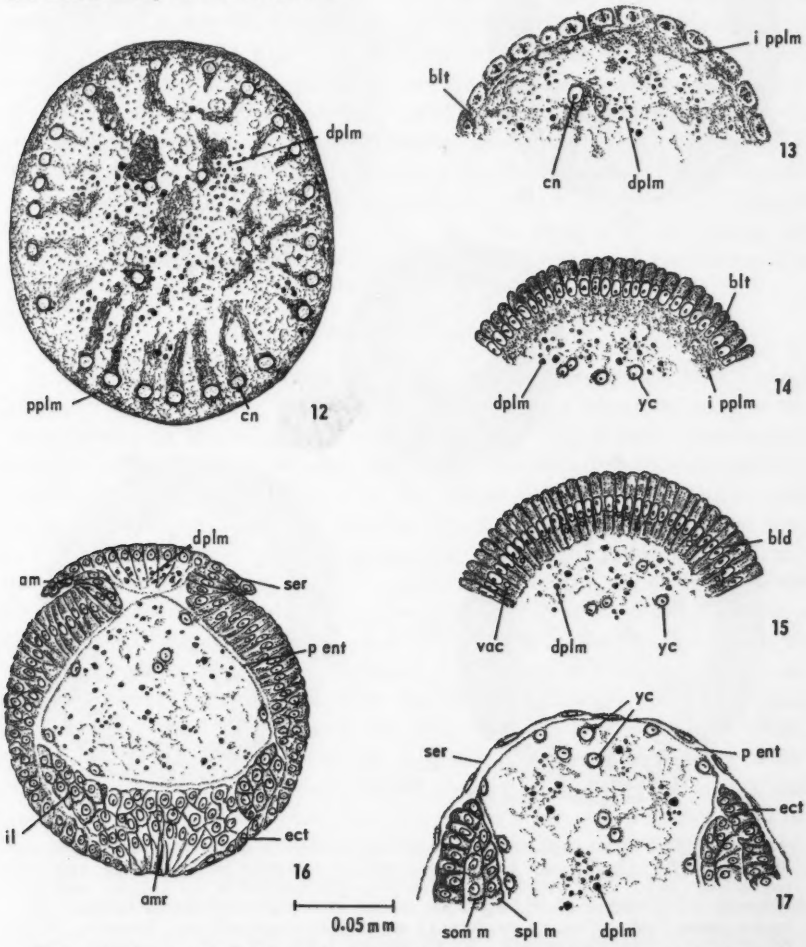
Embryonic Envelopes

Though insect embryos generally become enveloped by two embryonic membranes (serosa and amnion), one or both are often rudimentary, or entirely lacking, among the Hymenoptera. Both membranes appear to be lacking in some chalcidoids (29–31) and in the few ichneumonids studied embryonically (5, 38, 49). One embryonic envelope has been observed in the chalcidoids *Pseudaphycus* sp. (27) and *Eurytoma aciculata* (35). However, a well-developed serosa, which secretes a cuticular layer, and a rudimentary amnion are present in the sawfly *Nematus ribesii* (52).

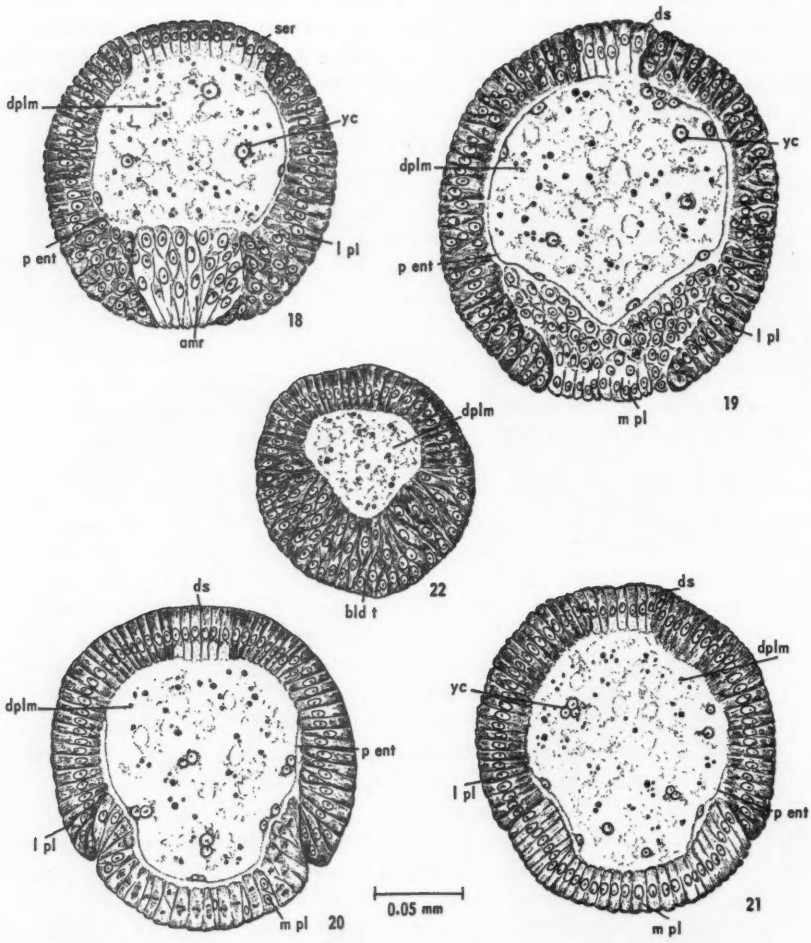
P. turionellae has a serosa, which entirely envelops the developing embryo (Fig. 9*a*, *ser*), and a rudimentary amnion. The amnion is transitory and is visible only in 9- to 10-hour embryos. The serosa develops from the extra-embryonic blastoderm, which begins to differentiate into the anterior serosal cap (*ser*) and the dorsal strip (*ds*) in 7-hour embryos. By 8 hours the concentration of the proximal cytoplasm of the anterior serosal cap and dorsal strip cells is greatly diminished. The basal cell membranes of these cells become indistinct, resulting in an extremely close association between the deutoplasm and their cytoplasm (Fig. 35). Several deutoplasmic granules (*dplm*) are visible within the proximal cytoplasm of the anterior serosal cap cells (*ser*) in 9-hour embryos (Figs. 16, 36). Deutoplasmic granules that become affixed within the cytoplasm of the prospective serosal membrane cells retain this association throughout embryonic development. They are clearly visible within the cytoplasm of the serosal membrane at the cephalic pole of advanced embryos (Fig. 37). Jackson (36) observed vesicles and refractive particles within the cytoplasm of the embryonic membrane in the braconid *Perilitus rutilis* (Nees.) These she considered to be nutritive substances absorbed from the host.

By 9 hours the anterior serosal cap cells (*ser*) have lost their original columnar shape and have begun to sever their attachment to the rudimentary amnion that develops as a transitory fold along the dorsal extremities of the germ band (Fig. 16). The serosal cells then begin to envelop the embryo within a membrane of simple squamous epithelium. The dorsal 'lifting up' of the serosal membrane from the deutoplasm proceeds caudad simultaneously with gastrulation (Figs. 6*a*, 6*c*). The posterior serosal cap, though slightly more tardy in developing than the anterior cap, is not so slow in forming as is the caudal fold in *A. mellifera* (42). While the serosal membrane is lifting away from the dorsal deutoplasm and the dorsal extremities of the germ band (Fig. 17), its anterior and posterior caps are enveloping the ventral surface of the embryo (Figs. 7*a*, 7*b*).

The cephalic and caudal serosal caps meet ventrad in the region of the fourth abdominal segment (Fig. 24). Usually the serosal membrane is completely formed in 12 to 13 hours. It remains intact throughout embryonal development. As in *E. aciculata* (35), the serosal membrane develops from the extraembryonic blastoderm.



FIGS. 12–17. *P. turionellae*. Camera lucida drawings of transverse sections of stained embryonic material. 12. At 3 hours, showing cleavage nuclei (*cn*) with protoplasmic 'tails' migrating to the periphery. 13. At 4 hours, a part of the peripheral region in the early blastemal stage. 14. At 5½ hours, a part of the blastema (*blt*). Cell membranes are beginning to appear. 15. At 6 hours, a part of the peripheral region, showing the completely formed blastodermal cells (*bld*). 16. At 9 hours through the anterior mid-gut rudiment (*amr*), showing the severance of the anterior serosal cap (*ser*) from the rudimentary amnion (*am*), and the deutoplasmic granules (*dplm*) lying within the serosal cells. 17. The dorsal region of an embryo at 10 hours, showing the formation of the primary entoderm (*p ent*) above the deutoplasm (*dplm*) as the serosa (*ser*) severs its dorsal attachment.



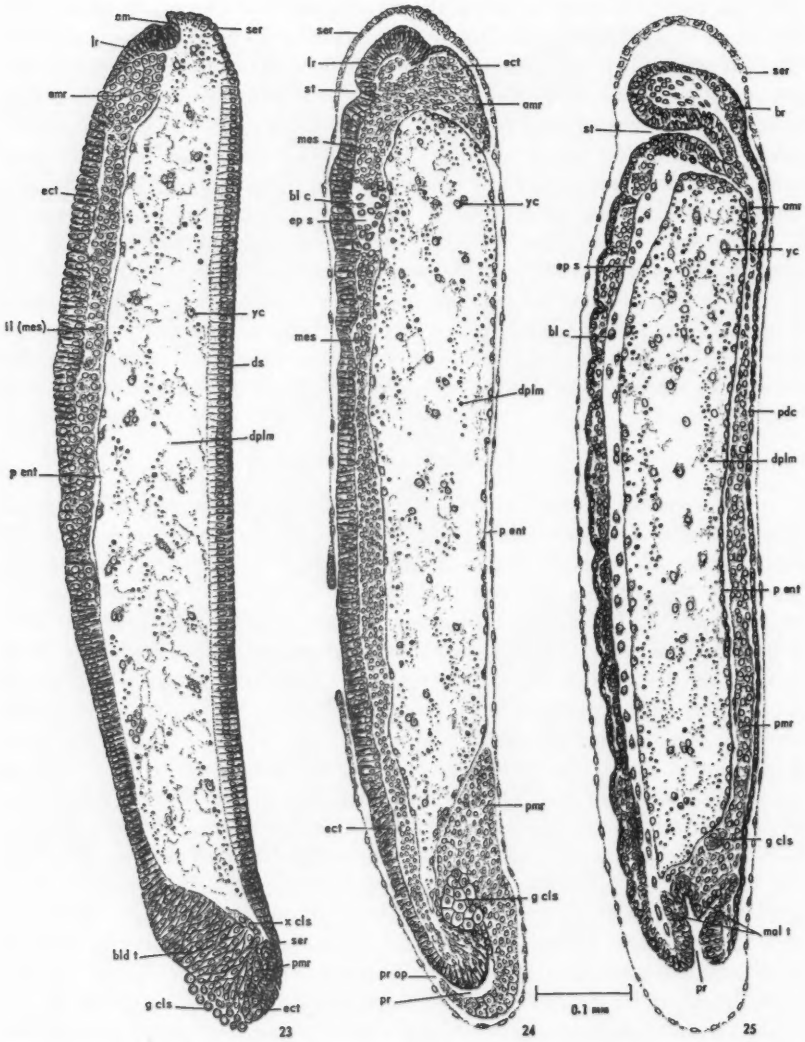
FIGS. 18–22. *P. turionellae.* Camera lucida drawings of transverse sections of an 8-hour embryo. 18. Through the anterior mid-gut rudiment (*amr*) and the anterior serosal cap (*ser*). 19. Through the middle plate just caudad of the anterior mid-gut rudiment. The middle plate (*m pl*) has thickened before submergence between the lateral plates (*l pl*). Primary entoderm (*p ent*) is present ventrally and laterally. The dorsal strip (*ds*) has differentiated. 20. Farther caudad than Fig. 19. Extensive mitotic activity has begun in this region of the middle plate (*m pl*), and the spindles are perpendicular to the surface of the embryo. Primary entoderm (*p ent*) is present ventrally and only partially formed laterally. 21. Just caudad of Fig. 20. The middle plate (*m pl*) is not, as yet, mitotically active. Primary entoderm (*p ent*) is beginning to appear lateroventrally. 22. Through the posterior blastodermal thickening (*bld t*). No primary entoderm is present, as yet, in this region.

Nelson (42) discussed in detail the development of the single embryonic membrane in *Apis mellifera*, which he referred to as the amnion on the authority of Bütschli (8). In *A. mellifera* the cells destined to form the embryonic membrane separate from the dorsal margins of the lateral plates. The 'amnion' grows mesiad over the dorsal strip, submerging it within the deutoplasm, where it appears to take part in the formation of the 'cephalodorsal body'. No such structure was observed in *P. turionellae*. Cephalic and caudal 'amniotic' caps develop in *A. mellifera* similar to the serosal caps in *P. turionellae*. They meet ventrad near the caudal pole to complete the embryonic membrane. When the 'amnion' peels from the dorsal yolk in *A. mellifera*, the yolk is left bare except for a thin 'protoplasmic pellicle' formed from the inner cortical layer, which has not taken part in the formation of the definitive blastodermal cells (42). At this time in *P. turionellae* the primary entoderm is differentiating on the dorsal surface of the deutoplasm (Fig. 17, *p ent*).

Primary Entoderm

In *P. turionellae*, the primary entoderm forms simultaneously with gastrulation. Figure 23 shows the presence of the primary entoderm only in the region where the inner layer has already formed. The primary entoderm is formed by the primary yolk cells. Several of the primary yolk cells that are concentrated centrally throughout the length of the deutoplasmic mass before gastrulation begin to migrate towards its periphery. Here they enclose the deutoplasm in a thin, sparsely nucleated, protoplasmic layer, the primary entoderm (*p ent*). It appears first in the region of the cleft between the middle and lateral plates (Fig. 21, *p ent*). Next it becomes differentiated above the middle plate (Fig. 20), and then in the region of the lateral plates (Fig. 19). It forms dorsally as the serosal membrane separates from the deutoplasm (Figs. 16, 17, 36). This nucleated membrane surrounds the deutoplasm during the remainder of embryonic development and is still visible after the mid-gut epithelium forms. The primary entodermal cells, remaining yolk cells, and deutoplasm are digested about the time of hatching. Cells accumulate at the anterior and posterior ends of the deutoplasm in association with the primary entoderm (Figs. 37, 41). They appear to be yolk cells and are digested with the primary entoderm.

The initial membrane surrounding the deutoplasm in *A. mellifera* develops from the inner cortical layer of protoplasm that is not involved in the formation of the definitive blastodermal cells. This layer of protoplasm is soon penetrated by yolk cells but remains as a definite structure until near the end of embryonic development (42). In *P. turionellae*, the entire cortical protoplasm (inner periplasm) becomes the proximal cytoplasm of the blastodermal cells (Figs. 14, 15). The primary entoderm of *B. femoralis* is formed by inner layer cells in association with formative protoplasm and possibly by some secondary yolk cells (5).



FIGS. 23–25. *P. turionellae.* Camera lucida drawings of sagittal sections of stained embryos. 23. At 9 hours through the median line, showing the formation of the inner layer (*il*) and the cephaloventral movement of the germ cells (*g cls*). 24. At 11½ hours through the median line. The inner layer (*mes*) is complete, and proliferation of the anterior and posterior mid-gut ribbons (*amr*, *pmr*) has begun. The cephalic and caudal serosal caps (*ser*) are meeting ventrally in the region of the fourth abdominal segment. 25. At 18 hours slightly laterad of the median line. The anterior and posterior mid-gut ribbons (*amr*, *pmr*) have met dorsally. The proctodaeal opening (*pr*) is caudal, and the Malpighian tubules (*mal t*) are evaginating from the anterior end of the proctodaeum.

Formation of Mid-gut Rudiments, Stomodaeum, and Proctodaeum

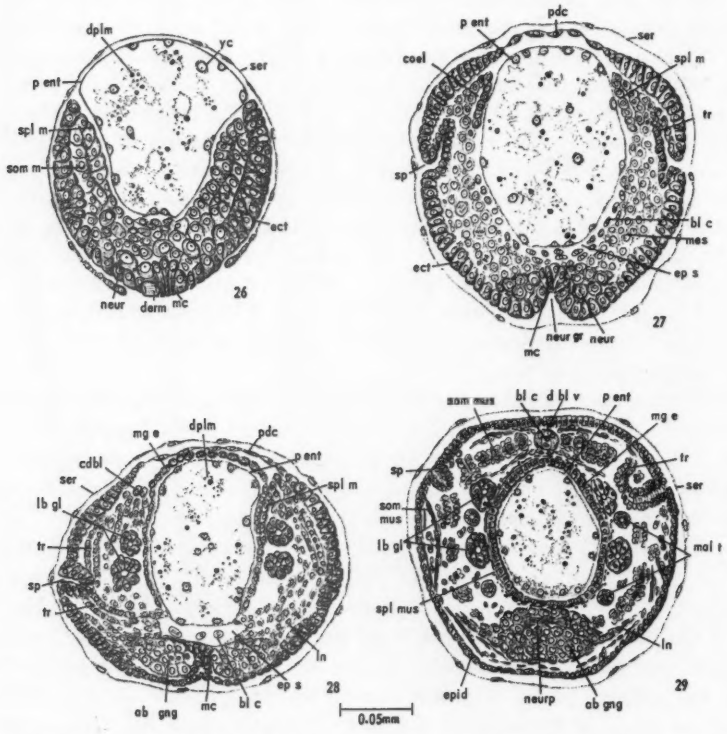
The mid-gut of *P. turionellae* is derived from two rudiments, which arise at the anterior and posterior ends of the germ band. The anterior mid-gut rudiment (secondary entoderm) initially differentiates from blastodermal cells at 7 hours, when a triangular darkened area is visible on the ventral mid-line of living embryos just in front of the middle plate (Figs. 3, 18). This newly differentiated mass of cells (*amr*) sinks beneath the ectoderm simultaneously with the mitotically active middle plate (Fig. 35), and only a small area of the anterior mid-gut rudiment remains exposed to the external surface (Figs. 16, 36). Later, this region becomes the floor of the stomodaeal invagination (Fig. 24, *st*), as in *A. mellifera* (42). Once beneath the ectoderm, the anterior mid-gut rudiment proliferates as a single concentrated ribbon over the anterior end of the deutoplasm, then caudad along its dorsal surface (Fig. 24, *amr*). It is in close association with the cephaloventral mesoderm (Fig. 24, *mes*) at this time. The anterior mid-gut ribbon thins out as it grows caudad (Figs. 25, 30, *amr*) and laterad (Fig. 28, *mg e*) over the surface of the deutoplasm. The invagination of the stomodaeum in the region of the anterior mid-gut rudiment begins in approximately 10-hour embryos (Figs. 6*a*, 6*b*, *st*). Its further invagination coincides with the proliferation of the anterior mid-gut rudiment (Fig. 25). By 18 hours the end of the stomodaeum has reached the cephalic end of the deutoplasm, and the anterior mid-gut tissue has covered its cephalic end like a cap (Fig. 37, *amr*, *st*). The stomodaeal and mid-gut lumina do not unite until late in embryonic development, when a proventricular valve similar to that found in *A. mellifera* (42) is formed at their junction.

The middle plate (mesoderm) and the anterior mid-gut rudiment (secondary entoderm) appear to form similarly. These tissues are differentiated at the same time in the ventral germ band and simultaneously sink beneath the ectoderm. But each remains as a distinct tissue, after its initial differentiation from the blastodermal cells.

As Nelson (42) observed few cell divisions during early development of the anterior mesenteron (mid-gut) rudiment in *A. mellifera*, he considered that it arises by immigration of the ventral blastodermal cells. He also noted that the mesenteron tissue with the exception of a circular area near its posterior border was soon covered by ectoderm. In *P. turionellae* there does not appear to be extensive mitotic activity in the anterior mid-gut rudiment as it sinks beneath the newly formed ectoderm. The anterior mid-gut rudiment cells seem to be pushed beneath the advancing ectodermal cells: the cone-shaped structure of these cells (*amr*) gives this impression, especially as their nuclei appear to be forced to their inner ends (Fig. 16). In *Chalicodoma muraria* (11) and *Ammophila campestris* (3) the mesenteron rudiments are formed by proliferation from the ventral blastodermal cells. In *Nematus ribesii* (52), mesendodermal rudiments are formed from which differentiate the mid-gut epithelium, the mesoderm of the procephalic region

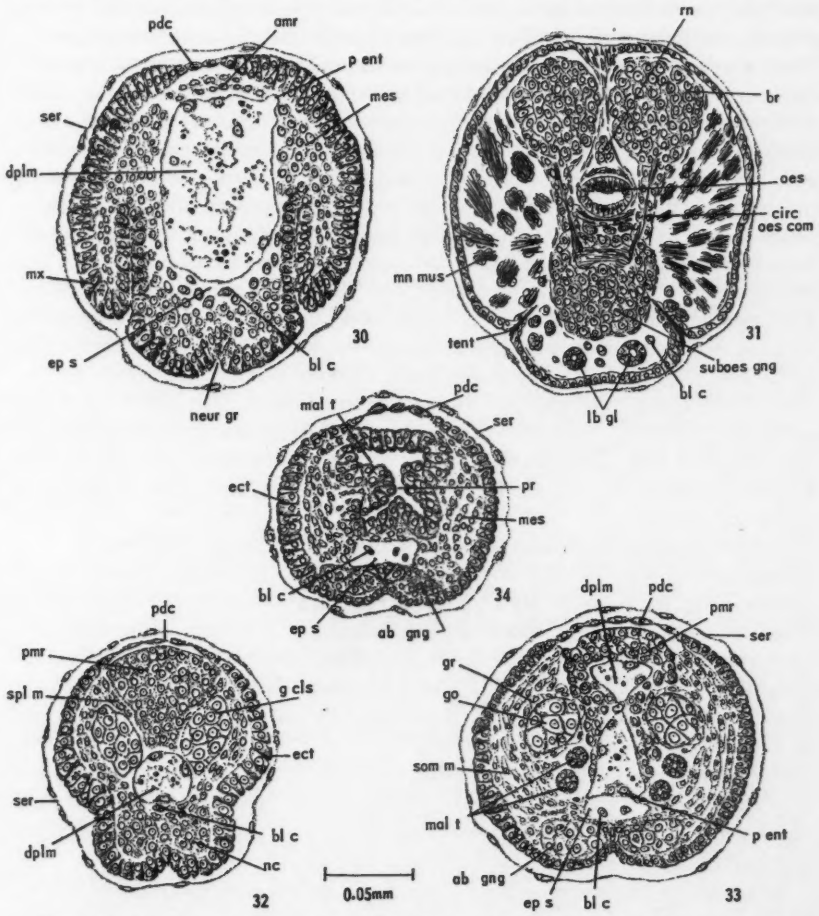
of the head, the muscle layer of the stomodaeum and proctodaeum, and the germ cells. Mesentodermal cells lying beneath the deutoplasm form the mid-gut epithelium in *Eurytoma aciculata* (35).

The formation of the posterior mid-gut rudiment and the proctodaeum is associated with the migration of the germ cells. The caudoventral blastodermal cells begin to thicken when the germ cells have reached the caudal pole in 5- to 6-hour embryos (Fig. 39, *bld t*). By 8 hours the caudoventral thickened area of the germ band is distinct (Fig. 22, *bld t*). This thickened area, the posterior mid-gut rudiment (*pmr*), begins to move dorsad as the dorsal strip (*ds*) and lateral plates (*l pl*) differentiate toward the caudal pole (Fig. 5). The germ cells (*g cls*), which project from the caudal pole in 7-hour



FIGS. 26–29. *P. turionellae.* Camera lucida drawings of stained embryos. 26. At 11 hours through the mid-abdominal region. The somatic mesoderm (*som m*) and splanchnic mesoderm (*spl m*) are distinct. Neuroblasts (*neur*) are differentiating, and the serosal membrane (*ser*) has nearly enveloped the entire embryo. 27. At 13 hours through the first abdominal segment, showing the coelomic cavities (*coel*) and tracheae (*tr*). The provisional dorsal closure (*pdc*) has completely covered the dorsal surface of the deutoplasm. 28. At 18 hours through the mid-abdominal region. The mid-gut tissue (*mg e*) has extended over the dorsal and lateral surfaces of the deutoplasm (*dplm*). The abdominal ganglia (*ab gng*) and epineural sinus (*ep s*) are well differentiated. 29. At 25 hours through the mid-abdominal region. The dorsal blood vessel (*d bl v*) has formed, and the epidermis is present dorsally.

embryos (Fig. 3), move ventrocephalad at the same time (Fig. 5). The clefts of the lateral plates (*cl l pl*) continue around the posterior end of the embryo to meet one another caudodorsally to the germ cells (Fig. 5). The germ cells in association with the posterior mid-gut rudiment (*pmr*) start



FIGS. 30–34. *P. turionellae.* Camera lucida drawings of stained embryos. 30. At 13 hours through the head in the region of the maxillary lobe (*mx*). The provisional dorsal closure (*pdc*) has formed above the anterior mid-gut ribbon (*amr*). 31. At 25 hours through the head in the region of the brain (*br*) and suboesophageal ganglion (*suboes gng*). 32. At 13 hours through the abdomen in the region of the germ cells. The provisional dorsal closure (*pdc*) has developed above the posterior mid-gut ribbon (*pmr*). The germ cells (*g cls*), now divided into two masses, lie dorsolaterally of the deutoplasm (*dplm*). 33. At 18 hours through the abdomen in the region of the gonads. Cells of the genital ridges (*gr*) have surrounded the germ cells forming the embryonic gonads (*go*). The posterior mid-gut ribbon (*pmr*) has extended ventrolaterally over the deutoplasm (*dplm*). 34. At 18 hours through the abdomen at the anterior end of the proctodaeum (*pr*). The four evaginations of the proctodaeum forming the Malpighian tubules (*mal t*) are distinct.

moving inward between the caudal folds of the lateral plates (Fig. 6*b*). As the germ cells continue to move inward, a lumen is left in their wake. This becomes the proctodaeal invagination, which at this stage is continuous with that of the posterior mid-gut rudiment (Figs. 24, 41, 42, *pr*). Thus, the proctodaeum is formed as a result of the cephalodorsal movement of the posterior mid-gut rudiment and the ventromesial union of the lateral plates. The inner layer (mesoderm) is completed caudally after the ventral union of the lateral plates at the posterior end of the embryo (Fig. 41, *mes*). Cells at the caudal tip of the blastodermal thickening (Fig. 40, *ect*) are prospective ectoderm resulting from the caudal extension of the lateral plates. The germ cells accompany the posterior mid-gut rudiment as it moves cephalad and sends a ribbonlike process from its anterior extremity above the deutoplasm (Figs. 32, 42, *pmr*). As it develops cephalad, a similar tissue proliferates from the anterior mid-gut rudiment. These two ribbons meet and fuse to form a continuous band from one mid-gut rudiment to the other. The ribbon from the posterior rudiment develops faster than that from the anterior rudiment so that fusion occurs in the third thoracic segment at 16 to 17 hours. Figure 25 illustrates these ribbons at the 18th hour, shortly after their union. As the two ribbons grow toward each other they also proliferate laterally, and then ventrally. By 20 hours the mid-gut tissue has extended over the ventral region of the deutoplasm, completing the mid-gut epithelium. Figure 29 shows the completed mid-gut epithelium in a 25-hour embryo.

As the posterior mid-gut ribbon progresses over the dorsal surface of the deutoplasm, the protodaeal opening moves caudad from its original ventral position (Fig. 42, *pr op*). By 14 hours it reaches its definitive caudal position (Figs. 8*a*, 43). In the ichneumonid *Exidechthis canescens* (49), the germ band extends dorsad over the caudal pole of the embryo with the protodaeal opening lying on the dorsal side about one-third the length of the embryo from the caudal pole, and in the first instar larvae the anal opening is still in a somewhat dorsal position above the tail. In *Apis mellifera* (42) the posterior mesenteron (mid-gut) rudiment moves from an initial ventrocaudal to a caudodorsal position before it proliferates, as a result of the extension of the germ band over the posterior pole. At first the proctodaeal invagination is dorsal in *A. mellifera* (42) and *Nematus ribesii* (52).

The mid-gut rudiments extend as single or paired ribbons over the surface of the deutoplasm during the formation of the mid-gut in embryos of different species of Hymenoptera. As in *P. turionellae*, single anterior and posterior ribbons develop above the deutoplasm in *A. mellifera* (42), whereas paired anterior and posterior ribbons extend along the ventrolateral deutoplasm in *N. ribesii* (52). A single posterior ribbon and paired anterior ribbons develop above the deutoplasm in the sphecid *Ammophila campestris* (3).

Bledowski and Krainska (5) noted that the 'pole caps' (prospective entoderm) were involved in the formation of three sections of the alimentary canal in *Banchus femoralis*. These 'pole caps' are formed from the surplus cleavage

nuclei that accumulate at either pole of the embryo after the blastoderm forms. As well as taking part in the formation of the dorsal mid-gut epithelium, they produce the 'stomodaeal mid-gut' and the 'proctodaeal mid-gut' regions of the alimentary canal that lie between the true stomodaeum (ectoderm) and the mid-gut, and between the proctodaeum (ectoderm) and the mid-gut, respectively. In this ichneumonid, mid-gut epithelium is also derived from cells in the mid-ventral region of the inner layer. The 'stomodaeal' and 'proctodaeal' mid-guts do not acquire a cuticular intima as they differ in origin from the true stomodaeum and proctodaeum. No such regions of the stomodaeum and protodaeum, derived from prospective entoderm tissue, were observed in *P. turionellae.*

Development of Gonads

The germ cells of *P. turionellae*, as noted previously, are polynuclear in origin. They are precociously segregated at the caudal pole during blastoderm formation.

The germ cells return within the embryo during gastrulation. Their position changes from polar to caudoventral as they become associated with the caudoventral thickening of the blastoderm (posterior mid-gut rudiment) (Fig. 23, *g cls*). The germ cells then invaginate with the posterior mid-gut rudiment as it sinks between the lateral plates, which are completing their ventromesial union just cephalad to them (Figs. 6*b*, 7*b*, *l pl*, *g cls*). The proctodaeal invagination forms as the germ cells move into the embryo, and this lumen is continuous with the invagination of the posterior mid-gut rudiment at this time (Figs. 41, 42). A similar inclusion of the pole (germ) cells within the invagination of the posterior mid-gut rudiment has been observed in *Drosophila melanogaster* (15, 59).

Aboim's (1) experiments on irradiated pole cells of *D. melanogaster* showed that the entire movement of these cells during embryonal development is passive. This seems to be the case with *P. turionellae*, as the mass of germ (pole) cells remains associated with the posterior mid-gut rudiment and appears to be carried cephalad into the body cavity with this rudiment during its dorsal proliferation above the deutoplasm (Figs. 41, 42). With the approach of the germ cell mass toward the posterior end of the deutoplasm, the proctodaeal opening changes from a ventral to a caudal position, and the proctodaeal wall closes in behind the germ cells (Figs. 42, 43). The germ cells pass similarly from the proctodaeal lumen to the body cavity in *Lucilia caesar* (L.) (16), in which a definite canal in the gut wall is formed through which the pole cells migrate. In *Melophagus ovinus* (L.) (39) and *Drosophila melanogaster* (59), the pole cells enter the body cavity by passing intercellularly through the gut wall. At 12 hours in *P. turionellae*, the germ cell mass has reached the dorsocaudal end of the deutoplasm and appears to push the deutoplasm slightly ventrocephalad in this region (Fig. 42). At this time the germ cells divide into two masses that lie somewhat laterad between the posterior mid-gut rudiment (Fig. 32, *pmr*) and the deutoplasm (*dplm*).

Between 18 and 20 hours, they reach their definitive embryonic position in the 10th body segment (Fig. 47, *go*), where they become embedded within the dorsolateral splanchnic mesoderm (the genital ridges), which forms a thin epithelial layer around them (Fig. 33, *gr*). The germ cells in association with the genital ridges develop into the larval sex organs. Though the gonads lie dorsolaterad in the 10th body segment of the first instar larva, this position is not universal among ichneumonids. Rietra (49) observed the gonads of the first instar larva lying between the 12th and 13th body segments in *Exidechthis canescens*.

The germ cells have been identified at various stages during the embryonic development of monembryonic Hymenoptera. Nelson (42) and Baerends and Baerends-van Roon (3) were unable to distinguish the reproductive rudiments until they appear in the region of the genital ridges. According to Shafiq (52), the germ cells arise from the posterior mesendodermal rudiment in *Nematus ribesii*. He noticed them in the dorsal region of this rudiment in 15-hour embryos. By 28 hours this germ cell mass divided to form two groups of cells that soon became embedded in the dorsal part of the mesodermal tubes, which differentiated into the genital ridges by 36 hours. Bledowski and Krainska (5) failed to discover the germ cells in the young embryo of the ichneumonid *Banchus femoralis*. However, they were able to distinguish the gonads in the lateral mesoderm after the mid-gut and silk gland elements differentiated. In the monembryonic chalcidoid *Trichogramma evanescens*, Gatenby (21) noticed the oösome at the caudal pole of the newly laid egg and the polynuclear origin of the germ cells. These cells were situated at the ventral edge of the caudal pole, and as they moved passively within the embryo during gastrulation they became surrounded by blastodermal (somatic) cells to form a germ cell pocket. This pocket remained medioventrally beneath the proctodaeum at the caudal end of the embryo. Gatenby observed six to seven germ cells in the pocket of *T. evanescens* and noted that they moved only a short distance as compared with embryos of other species of insects. In *Caraphractus reductus* (31), *Eurytoma aciculata* (35), and *P. turionellae* the germ cells differentiate, when the blastoderm forms, and sink into the posterior end of the embryo during gastrulation. Later the germ cell mass divides into two parts that come to lie on either side of the hind-gut. In some *P. turionellae* embryos studied, a few free cells from the germ cell mass appear to pass through the posterior blastodermal thickening before the germ cells move in mass into the embryo (Fig. 40, *x cls*). If this is true for all *P. turionellae* embryos, the fate of these cells is as yet unknown. They may form part of the cell mass that accumulates in the caudal primary entoderm (Fig. 41) and may be considered to be tertiary yolk cells. If this is the case, they do not take any part in the actual formation of the embryo, as these cells are digested with the rest of the primary entoderm about the time of hatching. A similar interblastodermal migration of free pole cells was observed in *D. melanogaster*, in which they are considered to be tertiary yolk cells by Rabinowitz (48). Poulson (45, 46) was able to study the fate of

these cells in *D. melanogaster* with the aid of the Bodian silver impregnation technique, which clearly differentiates the pole cells from other tissues. He found that these 'tertiary yolk cells' accompanied by the 'lost pole cells' (pole cells that enter the embryo through the posterior mid-gut invagination but that do not take part in the formation of the gonad as do many of the pole cells that enter in this manner) become associated with the anterior end of the posterior rudiment. They move cephalad with the posterior mid-gut ribbon and form part of the middle section of the definitive mid-gut epithelium. Poulson and Sonnenblick considered that pole cells of *D. melanogaster* are 'potential germ cells', as only some of them take part in the formation of the gonads. With the aid of more specific staining techniques for germ cells suggested by Chiquoine (12), Poulson (44), and Tawfik (61), a similar situation may be revealed in *P. turionellae* in which the 'germ cells', as discussed above, may be only 'potential germ cells'.

Embryonic studies on monembryonic Hymenoptera have revealed little detailed information on the migration of the pole (germ) cells, whereas in the Diptera the pole cells have been observed to enter into the body of the developing embryo by two definite methods: (1) by the passage between the posterior blastodermal nuclei or cells, and (2) by inclusion in the posterior invagination. The former method occurs in several primitive Diptera, including *Chironomus dorsalis* Mg. (41), *Simulium pictipes* Hgn. (20), and *Sciara coprohila* Lint. (9), and in the calliphorid *Phormia regina* (Mg.) (2). According to Fish (19), the pole cells enter the calliphorid *Phaenicia sericata* by the latter method. In the hippoboscid *Melophagus ovinus* (39), the pole cells that migrate first move directly into the posterior proliferation; the remainder enter by the posterior invagination. The pole cells enter the embryo of *D. melanogaster* by interblastodermal passage and by inclusion in the posterior invagination (47). This also appears to be the case in *P. turionellae*, though evidence of the interblastodermal passage of the pole (germ) cells is still inconclusive.

Formation of the Head

Though in parasitic Hymenoptera the head of the larva is often degenerate, the head capsule of the first instar *P. turionella* larva is a fairly heavily sclerotized, definite structure (Fig. 11*a*). The formation of the head is very conspicuous during ontogeny, with one-third of the germ band forming head tissue.

The number of segments that enter into the composition of the insect head has been controversial for many years (10, 14, 17, 37, 50). Though the head was observed developing in living *P. turionellae* embryos, it was difficult to ascertain which of the many grooves, differentiated shortly after the germ band formed, demarcated segments. The concept of head segmentation in arthropods as presented by Snodgrass (56, 58) appears to apply to *P. turionellae*: that the head consists of an acron bearing the eyes, first antennae, stomodaeum, and labrum; and four segments, the intercalary, mandibular, maxillary, and labial (Figs. 7–11). However, Butt (10) recently

considered that the intercalary (premandibular) segment in the postembryonic stages included the clypeus and labrum, the latter representing the fused appendages of this segment.

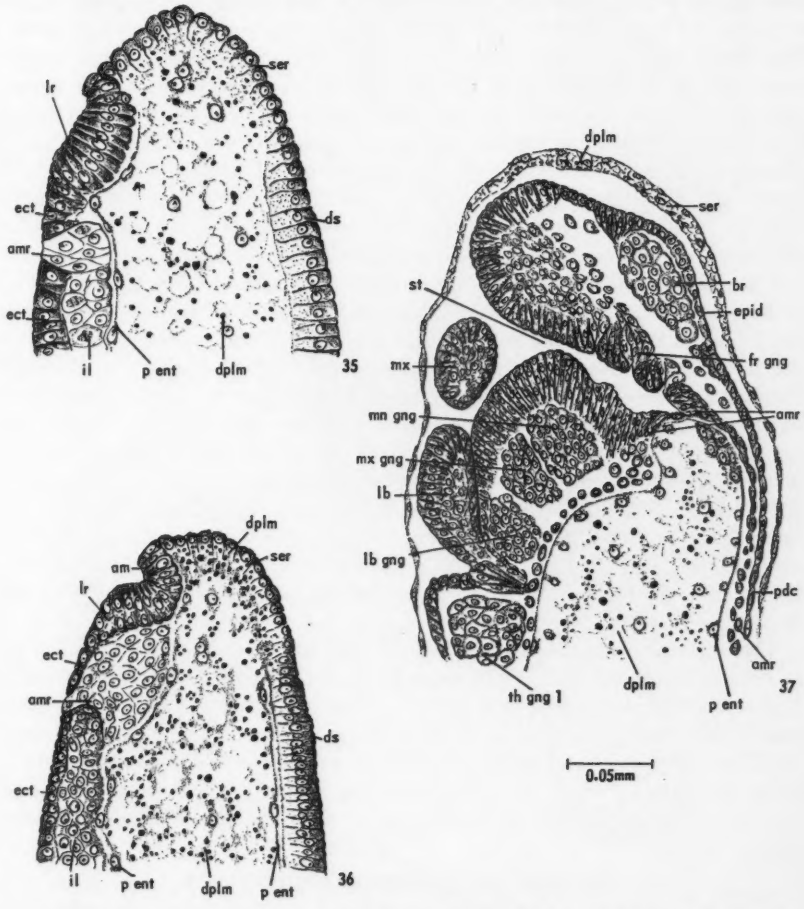
The first external indication of head development occurs in 7-hour embryos with the appearance of the maxillary segment (Fig. 3, *mx*) in the newly differentiated middle (*m pl*) and lateral plates (*l pl*). At this time the anterior mid-gut rudiment (*amr*) differentiates just cephalad to the middle plate, and the anterior cleft appears at the cephalic pole separating the germ band from the extraembryonic blastoderm (serosa and dorsal strip). The median ectodermal tissue lying cephalad to the anterior mid-gut rudiment thickens and elevates slightly, forming the labral lobe (Fig. 4, *lr*). The laterocephalic extensions of the clefts of the lateral plate demarcate the laterocaudal extremities of the labrum. The labrum also has a single origin in *Apis mellifera* (51), but a bilobed one in *Nematus ribesii* (52). By 8 hours the mandibular (*mn*) and labial (*lb*) segments are demarcated in the region of the middle and lateral plates (Fig. 4). During the ventromesial union of the lateral plates and the sinking-in of the anterior mid-gut rudiment, the labrum continues its development, appearing as a prominent unilobular structure at the anterior end of the germ band (Fig. 5, *lr*). In the next hour the serosal cap severs its attachment to the rudimentary amnion at the anterior end of the germ band and begins to envelop the head region (Figs. 6*a*, 7*a*, 16, *ser*). Now the antennal lobes differentiate. They appear postorally (Fig. 6, *ant*), as with *A. mellifera* (42) and *N. ribesii* (52), though their embryonic relationships are preoral. Coelomic sacs developed in the antennal region of *Apis mellifera* (42) and *Ammophila campestris* (3); these were not observed in *Nematus ribesii* (52) or in *Pimpla turionellae*.

After the ventromesial union of the lateral plates in 10-to-11-hour embryos, the intercalary segment (*intcal*), the gnathal lobes (*mn*, *mx*, *lb*), and neural crests (*neur cr*) differentiate (Figs. 7*a*, 7*b*). The gnathal lobes have a mesodermal core whereas the intercalary segment and neural crests become filled with neurogenic tissue. Both Nelson (42) and Shafiq (52) noted the extensive neurogenic nature of this intercalary (premandibular) region and agreed that it was not appendicular. Because of this, Shafiq considered that the premandibular segment is undeveloped in *N. ribesii*.

After the dorsomesial union of the ectodermal tissue over the anterior end of the deutoplasm, the labrum projects farther cephaloventrad and acquires a cephalomedian cleft (Figs. 7*b*, 7*c*). This development is accompanied by an invagination of the ectodermal tissue in the region of the stomodaeum.

Two general movements are involved in the further development of the head: a mesiocaudal advance of the dorsal ectoderm, and a mesiocephalic migration of the gnathal lobes on the ventral side of the embryo. During the latter movement the gnathal lobes meet ventrally below their neural crests as the latter develop into the suboesophageal ganglion. At this time the intercalary tissue (*br* III) begins to split mesially as it moves laterocephalad

around the stomodaeum (Fig. 8*b*). It soon sinks beneath the labrum (Fig. 9*b*) and becomes part of the definitive brain. The labial lobes fuse ventromesially and the line of fusion is soon lost (Fig. 10*b*). As the ventromesial movement continues, the labium is pushed cephalad and the maxillary lobes unite for a short distance behind it (Fig. 11*b*). The mesiocephalic movement of the gnathal lobes pushes the stomodaeal opening to a cephalic position, resulting in the formation of a definitive prognathic head.



FIGS. 35–37. *P. turionellae.* Camera lucida drawings of median sagittal sections through the anterior end of stained embryos. 35. At 8½ hours, showing the differentiation of the anterior mid-gut rudiment (*amr*), the inner layer (*il*), and the labrum (*lr*). 36. At 9 hours showing the presence of the deutoplasmic granules (*dplm*) in the cells of the serosal cap (*ser*). The labrum (*lr*) and the anterior mid-gut rudiment (*amr*) are further developed than in Fig. 35. 37. At 18 hours the brain tissue (*br*) has differentiated, the stomodaeal invagination (*st*) has reached the deutoplasm, and the ganglia of the gnathal lobes (*mn gng, mx gng, lb gng*) are fusing to form the suboesophageal ganglion.

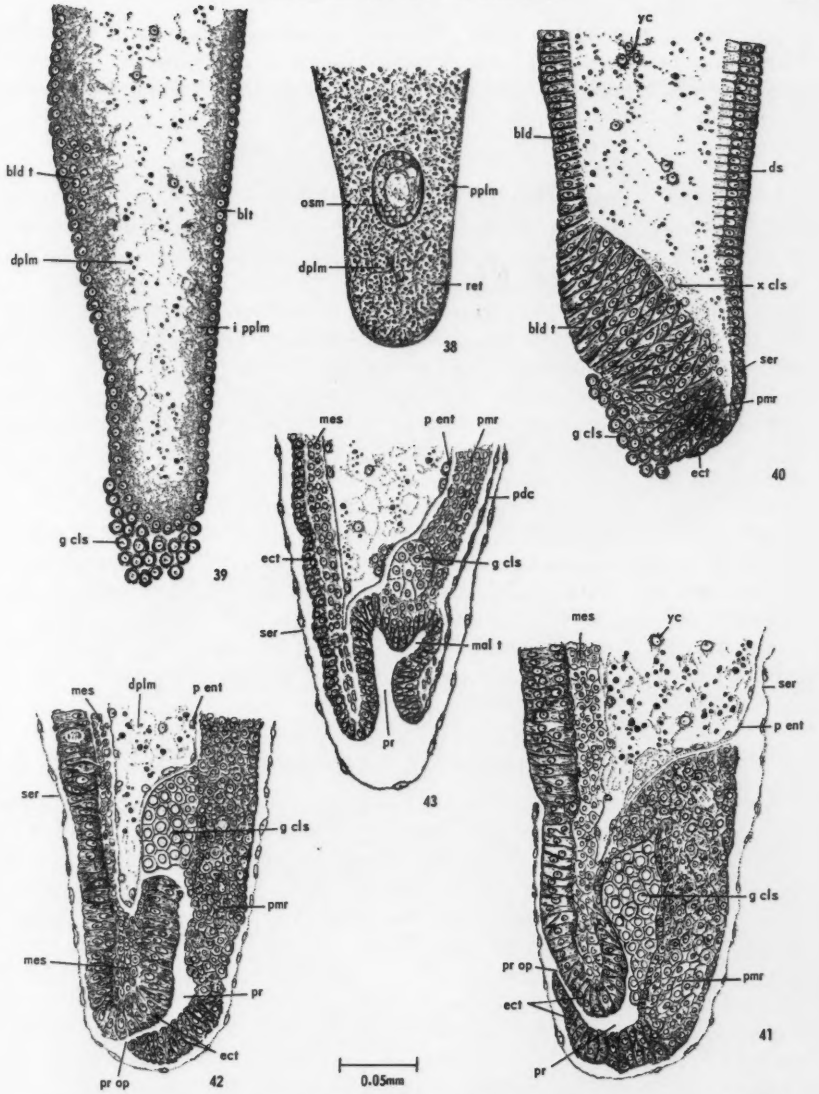
The protocerebrum (*br* I) and deutocerebrum (*br* II) develop from the dorsolateral ectoderm (Fig. 8*c*). By 18 hours the neurogenic tissue (*br*) has severed its ectodermal association, as the epidermal layer (*epid*) differentiates above it (Fig. 37). At this time the frontal ganglion (*fr gng*) is visible, arising from the dorsal wall of the stomodaeum (Fig. 37). By 22 hours the dorsal ectoderm has differentiated into its definitive dorsal areas, the clypeus and the frons (Fig. 9*a*, *clp*, *fr*). With the formation of the head capsule in 29-hour embryos the dorsomesial line of fusion is lost except at the dorsocaudal extremity of the head, where it remains as the ecdysial line (Fig. 11*c*, *ecdl*). The dorsal grooves, which extend from the antennal sockets to the dorsomesial line of fusion in 15-hour embryos, persist throughout embryonic development. In the first-instar head capsule the anterior arms of the ecdysial line occupy a somewhat similar position to these grooves (Fig. 11*c*, *aa ecdl*). The antennae move dorsad as the head develops. During the latter part of embryonic development, as their neurogenic associations develop internally (Fig. 46, *ant*), they degenerate externally, remaining as a pair of tiny unilobular sensory organs in the cephalolateral region of the head (Fig. 11*c*).

After the gnathal lobes differentiate, the labial glands (*lb gl op*) appear as ectodermal invaginations, one caudomesiad of each labial lobe (Fig. 7*a*). By 13 hours they become bilobed and extend into the thoracic region. In the ichneumonid *Banchus femoralis*, Bledowski and Krainska (5) observed the ducts of these glands to be ectodermal in origin but the glands themselves to be mesodermal, arising from lateral parts of the inner layer. During the remainder of embryonic development the labial glands proliferate caudad in *P. turionellae* as two bilobed ectodermal glands that lie laterad of the midgut (Fig. 29, *lb gl*). In the definitive embryo the two labial glands become bilobed in the third thoracic segment. Each lobe, consisting of basophilic, simple cuboidal epithelium, extends to the caudal end of the embryo. Within the head the two labial ducts fuse caudad of the labial gland opening (Fig. 46, *lb gl dt*).

The skeletal elements of the head are also ectodermal in origin and arise as tubular invaginations at the same time as the labial glands differentiate. Later they become lined with cuticle. The mandibular apodemes develop somewhat caudad of each mandibular base. The anterior tentorial arms invaginate just cephalad of the mandibular lobes, and the posterior tentorial arms arise directly above the labial lobe (Fig. 8*a*, *ptp*). The internal skeleton of the head in *P. turionellae* develops as in *Apis mellifera* (42) and *Nematus ribesii* (52).

Thoracic and Abdominal Appendages

Though rudimentary thoracic and abdominal appendages were observed in embryos of *P. turionellae*, they are transitory. They appear as ectodermal thickenings laterad of the neural crests and are distinct in 14- to 23-hour embryos (Figs. 8, 9). Three thoracic and nine abdominal appendages were observed, the thoracic ones being the more distinct. Bütschli (8) observed both thoracic and abdominal appendages in *Apis mellifera*, but Nelson (42)



FIGS. 38–43. *P. turionellae.* Camera lucida drawings of sagittal sections through the caudal end of stained embryos. 38. A newly laid egg through the median line, showing the oösome (*osm*). 39. At 5 hours through the median line. The germ cells (*g cls*) project from the caudal pole, and the caudoventral blastodermal thickening (*bld t*) has begun to form. 40. At 9 hours through the median line. The germ cells (*g cls*) have begun to move cephaloventrad. The 'X' cells (*x cls*) and posterior mid-gut rudiment (*pmr*) are visible. 41. At 11 hours through the median line. The germ cells (*g cls*) lie against the posterior mid-gut rudiment (*pmr*), and the proctodaeal invagination (*pr*) is distinct. 42. At 12 hours slightly laterad of the median line. The germ cells (*g cls*) are divided into two masses and lie in association with the posterior mid-gut ribbon (*pmr*) and the deutoplasm (*dplm*). Compare with Fig. 32. 43. At 14 hours through the right germ cell mass (*g cls*).

noted thoracic appendages only. Thoracic appendages have been found in *Ammophila campestris* (3) and *Nematus ribesii* (52). Wheeler (63) and Tanquary (60) noticed rudimentary appendages on the abdominal segments of ant embryos.

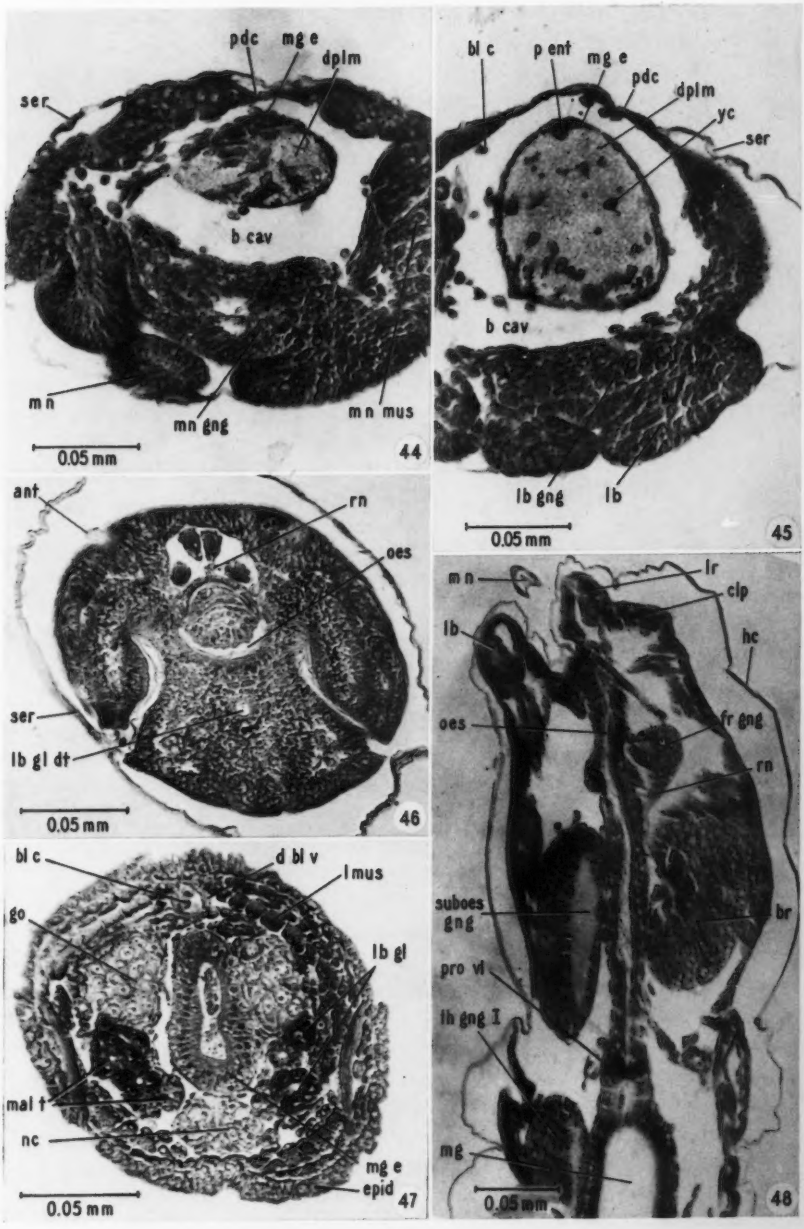
Dorsal Closure

When the serosa completely severs its dorsal attachment in 11-hour embryos of *P. turionellae*, only the primary entoderm covers its dorsal surface (Figs. 7*a*, 24, *p ent*). The mid-gut rudiments then begin to spread dorsad over the deutoplasm in their proliferation of the mid-gut epithelium (Figs. 24, 42). Soon, cells at the dorsolateral extremities of the ventral embryonic ectoderm begin to move dorsomesiad, covering the mid-gut tissue with a layer of simple squamous epithelium—the provisional dorsal closure. This tissue develops simultaneously at the anterior and posterior ends of the embryonic ectoderm (Figs. 30, 32, *pdc*). Though the provisional dorsal closure does not begin to develop until the mid-gut ribbons have progressed some distance over the deutoplasm, it quickly spreads and covers the dorsal surface of the embryo before the fusion of the anterior and posterior mid-gut ribbons (Figs. 8*c*, 27). More ectodermal cells move into the region of the provisional dorsal closure as lateral embryonic tissue continues its dorsomesial development above the deutoplasm. With the increase of ectodermal cells in this region, a simple cuboidal epithelial layer (Fig. 29, *epid*) is formed, replacing the provisional dorsal closure. A similar dorsal closure occurs in *Apis mellifera* (42) and *Ammophila campestris* (3). Nelson called this layer of simple squamous epithelium the extraembryonic ectoderm.

Mesoderm and its Derivatives

The mesoderm (inner layer), formed from the middle plate (*m pl*), thickens and then sinks beneath the lateral plates (*l pl*) of the germ band (Fig. 19). The inner layer is completed to the caudal extremity of the embryo by 11 hours. Immediately after the inner layer forms, its lateral regions differentiate into somatic and splanchnic mesoderm (Fig. 26, *som m*, *spl m*) with a distinct line of separation between them. By 13 hours a slitlike space is visible between these two layers. This narrow cleft represents the coelomic cavity (Fig. 27, *coel*) and appears to be continuous throughout the embryo. The coelomic cavities occupy a similar position in *Apis mellifera* and also are slitlike in form (42). Following the terminology of Carrière and Bürger (11), Nelson calls this lateral mesodermal region the mesodermal tubes.

FIGS. 44–48. *P. turionellae*. Photomicrographs of sections of stained embryos. 44. Transverse section at 18 hours through the head in the region of the mandibular segment (*mn*). 45. Transverse section at 18 hours through the head in the region of the labial segment (*lb*). 46. Transverse section at 27 hours through the head in the region of the antennae (*ant*). 47. Transverse section at 27 hours through the seventh abdominal segment, showing the gonad (*go*). 48. Median sagittal section at 28 hours through the head, showing the brain (*br*), frontal ganglion (*fr gng*), and the suboesophageal ganglion (*suboes gng*).



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Minute 'mesodermal tubes' are found in *Nematus ribesii* (52). Coelomic sacs are present in the antennal, thoracic, and abdominal segments of *Ammophila campestris* (3) but absent in *Eurytoma aciculata* (35). After the yolk shrinks, the epineural sinus develops dorsally above the mesial mesoderm. It appears in the gnathal region in 11- to 12-hour embryos (Fig. 24, *ep s*) and soon progresses caudad. Blood cells, differentiated from the mesial mesoderm, are visible scattered in the newly formed epineural sinus (Fig. 26, *bl c*). Splanchnic mesoderm becomes associated with the mid-gut ribbon as it proliferates laterad over the deutoplasm and later forms the muscles of the mid-gut (Fig. 29, *spl mus*).

Cardioblasts become differentiated from the lateral extremities of the mesoderm as it moves dorsomesiad above the mid-gut ribbons (Fig. 28, *cdbl*), and meet mesiodorsally to form the dorsal blood vessel (Fig. 29, *d bl v*). At this time histogenesis of the fat tissue, and of the somatic and splanchnic muscle, is also occurring. The dorsolateral splanchnic mesoderm differentiates into the genital ridges, which become associated with the germ cells to form the definitive embryonic gonad (Figs. 33, 47, *go*). The coelomic cavities unite with the epineural sinus to form the definitive body cavity.

Ectoderm and its Derivatives

Various structures of the definitive embryo develop from the ectoderm, formed by the lateral plates of the germ band. After the ventromesial union of the lateral plates, the neural groove (*neur gr*) and crests (*neur cr*) become visible in the mid-ventral region of 11-hour embryos (Fig. 7*b*). Their development into the nervous system is similar to that in *A. mellifera* (42). By 11 hours the tracheal invaginations (*sp*) begin to appear in the lateral walls of the ectoderm (Figs. 7*a*, 27). The tracheae are formed as in *Apis mellifera* (42), *Ammophila campestris* (3), and *Nematus ribesii* (52). However, the tracheal anlagen of the ichneumonid *Banchus femoralis* develops as a solid strand of ectoderm, which later acquires a lumen. The four Malpighian tubules appear as separate evaginations at the anterior end of the proctodaeum in 13- to 14-hour *P. turionellae* embryos (Fig. 43, *mal t*). They soon proliferate cephalad as paired ducts along the lateral walls of the mid-gut (Fig. 29). In *Chalicodoma muraria* (11) and *Apis mellifera* (42), the Malpighian tubules open directly onto the external surface of the embryo for a short time, as they develop in the proctodaeal tissue before the latter begins to invaginate. The Malpighian tubules do not arise until the pupal stage in *Banchus femoralis* (5). In this ichneumonid an 'anal vesicle', which appears to have an excretory function during larval development, is formed by the posterior end of the mid-gut in association with the 'proctodaeal mid-gut' and true proctodaeum. The epidermis of the mature *P. turionellae* embryo appears as a layer of simple cuboidal epithelium (Fig. 29, *epid*). The cuticle is formed 2 to 3 hours before the embryo hatches. Ectodermal derivatives formed in the head region were discussed in the section on head development.

Discussion and Conclusions

Embryogenesis has been described in greater or lesser detail for several species of Hymenoptera including: the honeybee, *Apis mellifera* L. (42, 51); the sphecid *Ammophila campestris* Jur. (3); the sawfly *Nematus ribesii* (Scop.) (52); the ichneumonids *Banchus femoralis* (Thoms.) (5) and *Glypta rufiscutellaris* Cress. (13); and the chalcidoids *Trichogramma evanescens* Westw. (21), *Caraphractus reductus* R.-Kors. (31), and *Eurytoma aciculata* Ratz. (35). The embryology of each differs from that of the others in one or more respects. The chief resemblances between *P. turionellae* and these species are as follows.

Gastrulation is as in *A. campestris* and *E. aciculata*. There are 10 abdominal segments as in *G. rufiscutellaris*, *E. aciculata*, and *N. ribesii*. Both a serosa and a rudimentary amnion are present, as in *A. campestris* and *N. ribesii*. The development of the head, of the mid-gut rudiments and ribbons, and of the coelomic cavities are as in *A. mellifera*. The neural crests and tracheae develop as in *A. mellifera* and *N. ribesii*, and the labial glands and ducts are ectodermal in origin as in these two species. The Malpighian tubules are ectodermal evaginations of the proctodaeum as in *A. campestris*, *E. aciculata*, and *N. ribesii*. An early differentiation of the germ cells and their passive migration from the caudal pole to within the embryo during gastrulation occurs as in *T. evanescens*, *C. reductus*, and *E. aciculata*.

The embryogenesis of *P. turionellae* is more similar to that of the aculeate *A. mellifera* and the chalcidoid *E. aciculata* than to that of other Hymenoptera that have been studied, including the closely related species *B. femoralis*.

The fact that *P. turionellae* is a parasitoid has had little influence on its embryogenesis, though in some other parasitic insects embryonic development has been simplified and the embryo hatches before histogenesis of its larval tissues is completed (28–34).

Summary

Superficial cleavage in the egg of the parasitic ichneumonid *Pimpla turionellae* results in the formation of a blastula consisting of columnar blastodermal cells and a central yolk mass (deutoplasm) in which are dispersed the primary yolk cells. At this stage some of the cleavage cells become associated with the germinal cytoplasm (oösome) at the caudal pole and project from the posterior end of the embryo; these are the germ, or pole, cells.

The blastoderm differentiates ventrally and laterally into the germ band and dorsally into the extraembryonic blastoderm, from which develops the serosal membrane. It envelops the embryo and persists until the embryo hatches. A rudimentary and transitory amnion develops as a fold along the dorsal extremity of the germ band in young embryos. The germ band differentiates into a middle and two lateral plates. As the middle plate sinks beneath the lateral plates (ectoderm), to form the inner layer (mesoderm), segmentation of the three plates occurs, the grooves that demarcate the maxillary segment appearing first. At this time the primary entoderm forms from the primary yolk cells and is digested later with the remaining

yolk cells. The anterior and posterior mid-gut rudiments extend as single ribbons over the dorsal surface of the deutoplasm and engulf the remaining yolk to form the wall of the mid-gut. The anterior mid-gut rudiment forms the floor of the stomodaeal invagination. The proctodaeum develops ventrocaudally, as the germ cells return into the embryo at gastrulation. The germ cell mass becomes associated with the posterior mid-gut rudiment and appears to be carried into the body cavity with the mid-gut ribbon as it proliferates cephalad. It divides at the posterior end of deutoplasm, and the two masses continue cephalad to their definitive dorsolateral position in the 10th body segment.

An acron and four head segments differentiate from the anterior third of the germ band. Two general movements are involved in the development of the prognathic head: a mesiocaudal advance of the dorsal ectoderm and a mesiocephalic migration of the three gnathal lobes on the ventral side of the embryo. The labrum develops as a median lobe just cephalad of the anterior mid-gut rudiment. The antennae, a pair of conspicuous lobes during the early development of the head, become rather degenerate in the mature embryo. Rudimentary and transitory thoracic and abdominal appendages develop. The provisional dorsal closure that develops from the dorsolateral extremities of the embryonic ectoderm covers the dorsal surface of the deutoplasm after the serosa severs its dorsal attachment to the deutoplasm. No antennal coelomic sacs were observed, but continuous rudimentary coelomic tubes are present in the lateral mesoderm. The Malpighian tubules develop from the anterior end of the proctodaeum. During development the embryo retains the original cephalocaudal axis of the egg; however, immediately before eclosion it rotates 180° on its longitudinal axis.

Acknowledgments

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Abbreviations Used in Figures

aa ecdl, anterior arm of the ecdysial line
ab 8, eighth abdominal segment
ab 10, 10th abdominal segment
ab ap 1, first abdominal appendage
ab gng, abdominal ganglion
am, amnion
amr, anterior mid-gut rudiment
an, anus
ant, antenna
antr, anterior
ata, anterior tentorial arm
atp, anterior tentorial pit
b cav, body cavity
bl c, blood cell
bld, blastoderm
bld t, blastodermal thickening
blt, blastema
br, brain
br I, protocerebrum
br II, deutocerebrum
br III, tritocerebrum
cdbl, cardioblast
circ oes com, circumoesophageal commissure
cl l pl, cleft of the lateral plate
clp, clypeus
clplr sl, clypeolabral sulcus
cn, cleavage nucleus
coel, coelomic cavity
d bl v, dorsal blood vessel
derm, dermatoblast
dplm, deutoplasm
ds, dorsal strip
ecdl, ecdysial line
ect, ectoderm
ep s, epineural sinus
epid, epidermis
epst, epistoma
fr, frons
fr gng, frontal ganglion
g cls, germ cells
gast gr, gastrular groove
ge, gena
go, gonad
gr, genital ridge
hc, head capsule
hl, head lobe
hst, hypostoma
hyp, hypopharynx
i pplm, inner periplasm
il, inner layer
intcal, intercalary segment
l mus, longitudinal muscle
l pl, lateral plate
lb, labium
lb gl, labial gland
lb gl dt, duct of the labial gland
lb gl op, opening of the labial gland
lb gng, labial ganglion
lb plp, labial palp
lb s, labial sclerome
ln, lateral nerve
lr, labrum
m pl, middle plate
mal t, Malpighian tubule
mc, median cord
mes, mesoderm
mg, mid-gut
mg e, mid-gut epithelium
mi dt, micropylar duct
mi op, micropylar opening
mn, mandible
mn gng, mandibular ganglion
mn mus, mandibular muscle
mx, maxilla
mx cd, maxillary cardo
mx gng, maxillary ganglion
mx l, maxillary lobe
mx s, maxillary sclerome
mx st, maxillary stipes
nc, nerve cord
neur, neuroblast
neur cr, neural crest
neur gr, neural groove
neurp, neuropile
oes, oesophagus
osm, oösome
p ent, primary entoderm
pdc, provisional dorsal closure
plst, pleurostoma
pmr, posterior mid-gut rudiment
pplm, periplasm
poc, postocciput
pos, postoccipital suture
pr, proctodaeum
pr op, proctodaeal opening
prmt, prementum
pro vl, proventricular valve
ptmt, postmentum
ptp, posterior tentorial pit
ret, reticulum
rn, recurrent nerve
ser, serosa
som m, somatic mesoderm
som mus, somatic muscle
sp, spiracle
spl m, splanchnic mesoderm
spl mus, splanchnic muscle
st, stomodaeum
st op, stomodaeal opening
suboes gng, suboesophageal ganglion
tent, tentorium
th I, first thoracic segment
th III, third thoracic segment
th ap I, first thoracic appendage
th ap III, third thoracic appendage
th gng I, first thoracic ganglion
tr, trachea
vac, vacuole
vent, ventral
x cls, 'X' cells
yc, yolk cell

SOME EFFECTS OF OXYGEN IN RELATION TO TEMPERATURE ON THE DEVELOPMENT OF LAKE TROUT EMBRYOS[1]

E. T. GARSIDE[2]

Abstract

Lake trout embryos were exposed to dissolved oxygen levels approximating 2.5, 3.5, 4.5, and 10.5 p.p.m. at each of four temperatures, 2.5° C, 5.0° C, 7.5° C, and 10.0° C, from fertilization to a late stage of development. The three low oxygen levels caused retardations in the developmental rates resulting in delayed hatching and lengthening of the hatching period, reduction of vitelline circulatory structures, and abnormalities of head and trunk. The low oxygen levels at 10° C caused total mortality just prior to hatching. These results are discussed and compared with previous findings.

Introduction

Several investigators, among them, Battle (2), Embody (3), Gray (5), Hayes and co-workers (6, 7, 9), and Price (13) have called attention to the relationship of temperarure to embryonic development of salmonoids. Studies of critical levels of oxygen during early development have been carried out by Alderdice *et al.* (1), Hayes *et al.* (8), and Lindroth (12). The influence of oxygen on the morphology of salmonoids has been investigated by Alderdice *et al.* (1), Stockard (14), and Tåning (15).

Embryos of the lake trout *Salvelinus namaycush* (Walbaum) were used in the present study. The experiment was designed to study the effects of oxygen in relation to temperature on the rate of development and hatching, and on morphological characters of the resulting fish. Speckled trout were also reared in this experiment and the findings will be reported later when the morphological data are gathered. Suffice it to state that the developmental data parallel those of the lake trout.

Materials and Methods

Approximately 12,000 freshly fertilized lake trout eggs were obtained on October 22, 1957, from the Ontario Department of Lands and Forests during routine spawn-taking operations at Lake Simcoe, Ontario. The eggs were transported in a conventional egg carrier to the Laboratory for Experimental Limnology at Maple, Ontario, where they were divided into 16 approximately equal lots and placed in the experimental environments within 6 hours of fertilization.

The eggs were placed in circular trays, 6 in. outside diameter and 1 in. deep, made of aluminum frames covered with aluminum mesh. Two trays (one contained the speckled trout eggs) were placed in an incubation chamber

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made of a closed lucite cylinder 6 in. in diameter and 6 in. high. Water for the incubating eggs entered the chamber through a vertical aluminum tube which passed through the center of the chamber cover and through the centers of the two trays. Water passed through openings in the wall of the tube at the levels of the trays. After passing through the eggs, the water left the chamber through small perforations in the cover. Four chambers were attached linearly to an aluminum frame and were suspended in a water bath.

Temperatures of 2.5° C, 5.0° C, 7.5° C, and 10.0° C were maintained in the baths by mixing 9° C laboratory tap water with appropriate amounts of 1.5° C refrigerated water or by heating.

The gas exchange method as shown in Fig. 1 was used to maintain three levels approximating 2.5 p.p.m., 3.5 p.p.m., and 4.5 p.p.m. dissolved oxygen. Nitrogen was bubbled into refrigerated water falling through a 4-ft column of glass marbles. The partially deoxygenated water was divided into two flows one of which went directly to a fractionating column modified from Fry (4) and the other flow passed through a countercurrent heat exchanger and was warmed to 6° C before entering another fractionating column. The fractionating column with the colder water supplied the incubation chambers in the 2.5° C and 5° C baths while warmer water was supplied to the chambers

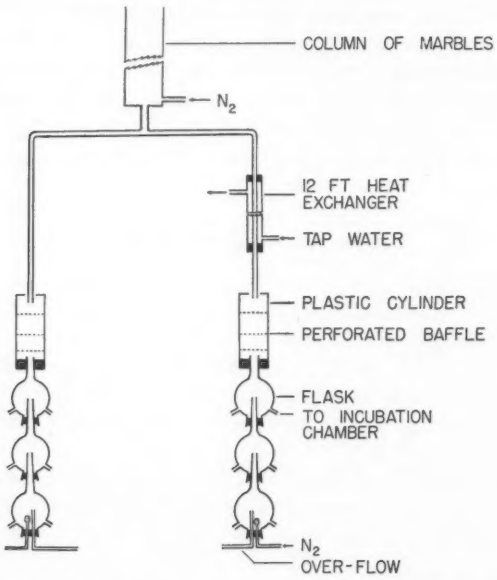

FIG. 1. Gas exchange apparatus for providing water of various dissolved oxygen content. Water falls through the column of glass marbles and thence into two fractionating columns. The column on the left supplies the incubation chambers in the 2.5° C and 5° C water baths and the other column supplies the chambers in the 7.5° C and 10° C baths.

in the 7.5° C and 10° C baths. To insure complete temperature equilibration, water from the flasks of the fractionating columns was passed through 12-ft lengths of coiled $\frac{1}{4}$-in. aluminum tubing submerged in the baths before it was directed into the incubation chambers. Chambers containing the control lots were supplied with tap water equilibrated to the appropriate temperatures by passing it through coils of aluminum tubing in the water baths. Approximately one liter per minute flowed through each of the 16 chambers.

Water temperatures in each chamber were measured twice daily and the mean temperature recorded. Dissolved oxygen levels were determined daily by the unmodified Winkler method. The mean daily temperatures and oxygen levels are shown in Table I. The number of days of exposure to low oxygen for each lot is also given there. Tap water equilibrated to the required temperatures was provided for all chambers when the low oxygen treatments were terminated. The temperature was maintained to the end of the hatching period for each lot of embryos. When hatching was completed the alevins were placed in aquaria at 9° C.

TABLE I

Experimental environments for incubation of lake trout eggs. Treatment with low oxygen was terminated at the times indicated when the embryos were in an advanced state of development

Mean temp., °C	Mean dissolved O_2, p.p.m.	Oxygen treatment in days	Mean temp., °C	Mean dissolved O_2, p.p.m.	Oxygen treatment in days
2.5	2.6	77	7.5	2.6	53
2.5	3.7	77	7.5	3.4	53
2.5	4.5	77	7.5	4.3	53
2.5	10.5	—	7.5	10.3	—
5.0	2.6	69	10.0	2.5	50
5.0	3.6	69	10.0	3.3	50
5.0	4.4	69	10.0	4.2	50
5.0	10.4	—	10.0	10.2	—

The progress of development of the embryos was followed by observation of samples of eggs collected at regular intervals and preserved in Davidson's fixative. Sampling was begun on day one, eggs incubated at 2.5° C and 5° C were sampled on alternate days and those at 7.5° C and 10° C were sampled daily. Five eggs were collected as a sample. Eighteen stages, listed in Table II, were used to follow the development of the various lots of embryos. The embryos of a sample were considered to have arrived at a specific stage when at least three of the five embryos exhibited the features of that stage.

With the onset of hatching the number of embryos which hatched each day in each lot was recorded and the median hatching times were determined. The number of days of incubation prior to the beginning of hatching plus the number of days taken to reach the median hatching day in the hatching period were considered as the incubation time.

TABLE II

Selected embryonic stages in the lake trout. Stages are based on external anatomical features evident in preserved embryos

No.	Stage	Description
1	Small blastodisc	Multicellular, polar cap, oblate–spheroid
2	Large blastodisc	Much flattened, thin, undifferentiated cap
3	Germ ring and embryonic shield	Peripheral band of cells beginning to overgrow yolk; inwardly directed mass of cells (the shield)
4	One-quarter epiboly	Germ ring has overgrown one-fourth of yolk; shield elongating
5	One-half epiboly	Germ ring has overgrown one-half of yolk; cephalic differentiation beginning
6	Three-quarters epiboly	Germ ring has overgrown three-quarters of yolk; somites differentiating
7	Blastopore closing	Small drop-shaped space within germ ring
8	Blastopore closed	Segmenting trunk ending in node
9	Trunk free of sac	Segmenting trunk has grown beyond and over sac, beginning to coil
10	Fin-fold	Fin-fold evident in middorsal line
11	Vitelline venation beginning	Vitelline vein beginning to grow laterally and posteriorly
12	One-quarter vitelline venation	Vitelline vein advancing cephalad over yolk sac, covering posterior quarter
13	One-half vitelline venation	Vitelline vein covering posterior half of yolk sac
14	Light eye pigment	Small pigmented areas around lens
15	Complete eye pigment	Iris completely pigmented
16	Notochord upturned	Posterior tip of notochord and vertebral column flexing caudodorsad
17	Caudal fin forming	Hypural elements forming plate; fin-fold taking on shape of caudal fin
18	Head and body pigment	Melanophores covering head and forming longitudinal lines along body

Results

The data are summarized in Tables III and IV. In every instance, after the first few days, the embryos reared in an hypoxial environment when compared with the appropriate control lot showed a delay in reaching a given stage and in hatching. The extent of the delay increased with the reduction of dissolved oxygen levels at each temperature.

The numbers of embryos that died during incubation were not recorded since it would be difficult to assess the amount of mortality which could occur from daily handling. From the time of blastopore closure to the turning of the notochord there was little mortality in any of the lots. Subsequently, however, there were substantial increases in mortality in the lots incubated at hypoxial levels and for this reason the oxygen treatments were discontinued. The three lots of embryos incubated in hypoxial conditions at 10° C did not respond to the increased oxygen supply and eventually all died before they were able to hatch, except for two embryos which hatched in the lot at 4.5 p.p.m. oxygen: these latter also died within a few hours of hatching. On the 11th day of the hatching period (day 91) the water supply to the 5° C control chamber became disconnected accidently and all embryos were killed. Up to this time 15 of the 388 possible embryos had hatched. During the 14 hours or less that the water supply was disrupted, 359 embryos had hatched and

died and only 14 embryos remained unhatched but these were dead also. A large number of the remaining embryos in each of the other 12 lots hatched successfully.

Vitelline vascularization was altered in the lots incubated at low levels of dissolved oxygen. Commonly the vitelline vein was interrupted in several places and the secondary vessels occurred in isolated patches. The degree of reduction of vascularization was greatest at 7.5° C and 10° C.

TABLE III

Times, in days, required by the various lots of lake trout embryos to reach each of the 18 stages listed in Table II. Columns are headed by mean dissolved oxygen levels expressed as p.p.m.

	2.5° C					5.0° C					7.5° C					10.0° C				
Stage	2.6	3.7	4.5	10.5	Stage	2.6	3.6	4.4	10.4	Stage	2.6	3.4	4.3	10.3	Stage	2.5	3.3	4.2	10.2
1	5	5	5	5	1	3	3	3	3	1	3	3	3	3	1	3	3	3	3
2	9	9	9	9	2	7	7	7	7	2	6	6	6	6	2	5	5	5	5
3	16	15	15	13	3	13	13	11	11	3	10	10	10	8	3	6	6	6	6
4	21	21	21	18	4	16	15	15	15	4	12	12	12	11	4	9	9	9	8
5	23	24	23	19	5	17	16	16	16	5	13	13	13	12	5	10	10	10	9
6	25	26	25	23	6	19	17	17	17	6	14	14	14	13	6	11	11	11	10
7	28	28	27	25	7	21	20	20	19	7	15	15	16	14	7	12	12	12	11
8	33	33	32	28	8	23	23	23	22	8	18	17	17	15	8	13	13	13	12
9	45*	44	41	35	9	29	27	26	25	9	23	23	23	18	9	19	19	18	15
10	51	49	49	45	10	38	35	34	33	10	26	26	25	22	10	23	22	20	17
11	57	55	51	50	11	42	37	37	35	11	29	28	26	23	11	26	24	22	19
12	65	61	61	55	12	46	43	43	37	12	32	29	29	25	12	27	27	25	20
13	69	65	65	61	13	48	45	45	41	13	33	30	31	28	13	28	28	27	21
14	76	71	69	65	14	54	49	47	45	14	37	34	33	31	14	32	30	30	25
15	79	75	75	69	15	58	55	51	47	15	41	40	37	34	15	38	33	32	26
16	87	81	79	76	16	64	65	59	53	16	46	44	42	38	16	40	36	34	28
17	103	95	93	87	17	80	79	71	64	17	57	55	52	46	17	42	40	40	32
18	113	100	99	95	18	84	82	76	70	18	60	58	55	50	18	47	43	42	34

TABLE IV

Lake trout hatching times given in days. Thermal units are based on calculated developmental zero, −1.5° C

Temp., °C	O_2, p.p.m.	Beginning of hatch	Period	Median day	Incubation time	Thermal units
2.5	2.6	129	36	27	156	624
	3.7	111	50	40	151	604
	4.5	128	30	22	150	600
	10.5	102	46	39	141	564
5.0	2.6	97	27	20	117	761
	3.6	94	22	15	109	709
	4.4	90	23	18	108	702
	10.4*	80	(11)	—	(91)	(591)
7.5	2.6	74	17	11	85	765
	3.4	72	16	11	83	747
	4.3	68	18	14	82	738
	10.3	60	12	7	67	603
10.0	2.5	–	–	–	(63)	(724)
	3.3	–	–	–	(63)	(724)
	4.2	61	–	–	(61)	(702)
	10.2	46	8	4	50	575

*Accidently lost on day 91.
Incomplete total, in parentheses, complete mortality.

Deformed embryos were found in all the lots incubated in hypoxial conditions. The frequency of deformities and their severity appeared to diminish from the 7.5° C lots to the 2.5° C lots. Figure 2 shows some of the abnormalities. Axial deformities such as those described by Alderdice *et al.* (1) were most frequent. Anomalous developments in the head region occurred in some of these embryos. Truncation of either or both jaws, microphthalmia, monophthalmia, and anophthalmia were found in several instances. There was no detectable reduction in eye pigmentation as reported in chum salmon (1) even in embryos with eye deformities.

Discussion

The data indicate that low ambient dissolved oxygen concentrations have profound effects on the velocity and extent of differentiation processes in the development of lake trout embryos. The first recognizable manifestation of hypoxia is a decrease in the normal rate of development. The degree to which development is retarded is directly related to the degree of hypoxia. Decelerated development begins at the onset of axial differentiation and continues through the remainder of the embryonic term if hypoxial conditions persist. Alderdice *et al.* (1) report that the period of maximum stress in chum salmon embryos is from 100 to 200 day-degrees Centigrade when the incubation temperature is 10° C. This period corresponds to the formation of the trunk. They suggest that subsequent reduction of stress results from the formation of a functional circulatory system which enhances the respiratory exchange between the embryo and the perivitelline fluid. No reduction in stress was found in lake trout embryos. However, the lake trout embryos were continuously exposed to low levels of oxygen and were unable to develop normal circulatory structures whereas the chum salmon embryos were exposed for only 7 days and the lots exposed prior to establishment of circulatory structures were not again exposed but new lots with circulatory structures were used to assess the effect of hypoxia on more advanced embryos.

The delay in hatching and extension of the hatching period are to be expected as natural consequences of the delay in development. Under certain circumstances, lowered oxygen can induce hatching at a time earlier than normal for a particular temperature. Alderdice *et al.* (1) exposed chum salmon embryos which had passed 77% of their predicted incubation time (452 day-degrees) to water of low oxygen content (0.8 p.p.m.) for 7 days. At the end of the exposure several of the embryos had hatched, about 6 days prematurely. Essentially this situation was produced accidentally in this experiment (see "Results", 5° C control lot). The reason for the acceleration of the hatching mechanism is unknown, although temporary, increased activity might be suggested as a partial explanation.

Hayes and co-workers (6, 7, 9) report the uncoupling of embryonic stages in the Atlantic salmon (*Salmo salar* Linn.). The term "uncoupling" is used in the sense that the sequence of embryonic features changes when the rate of development is altered. His considerations lead him to state (7), "As the

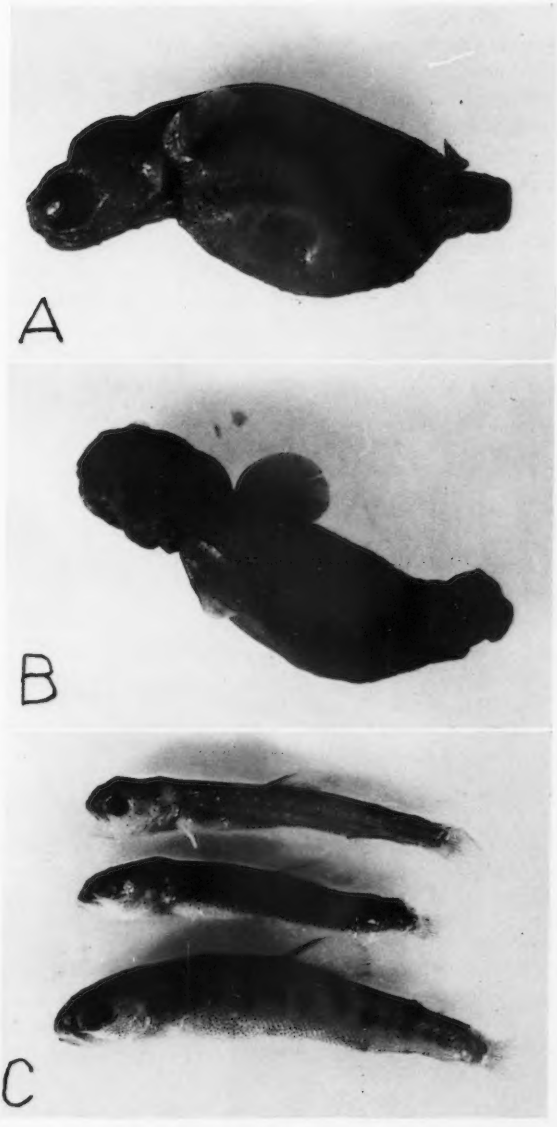

FIG. 2. A. Lake trout alevin incubated at 2.5 p.p.m. O_2, 7.5° C, showing developmental failure of the trunk. B. Dorsal view of alevin in A. C. Upper two lake trout alevins were incubated at 2.5 p.p.m. O_2, 2.5° C; they are in a state of starvation because of jaw deformities which inhibit feeding. The lower alevin was incubated at 10.5 p.p.m. O_2, 2.5° C, and is considered to be a normal fish.

question now stands it appears that the relationship of growth to differentiation is a matter considerably more complex than had been supposed and that the classical idea of an embryonic 'stage' will have to be abandoned". This phenomenon has not been demonstrated in lake trout embryos. Hayes observed three features, *completion of yolk circulation*, *establishment of tail circulation*, and *appearance of bile in lumen of gut*. Hayes' figure showing uncoupling of these features is reproduced in Fig. 3. The vagrant character is the bile in the lumen of the gut; the other characters, the circulatory structures of the yolk sac and tail, remain distinctly separate over the whole range of temperatures at which eggs were incubated. The secretion of bile should be considered a physiological phenomenon, not an anatomical character, and its use as a stage among anatomical characters is not justified. It is not suggested that the relationship of growth to differentiation is not complex but rather that it is still possible to make use of the classical idea of an embryonic "stage". Hayes' data indicate a complex relationship between anatomical and physiological differentiation.

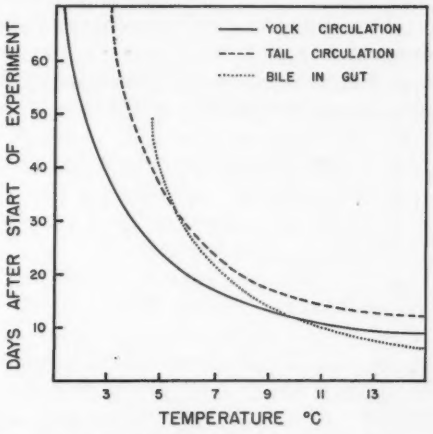

FIG. 3. Hayes' demonstration of uncoupling or change in sequence of embryonic features in Atlantic salmon. Reproduced from Hayes (7).

Hayes and Pelluet (6) state that the use of hatching as an embryonic stage is unsatisfactory because of its variability. Again, hatching is a physiological event, not an anatomical character, and it is for this reason that it should not be used as a stage with other features which are anatomical. However, normal lake trout embryos appear to hatch at the same anatomical stage.

An examination of the rule of thermal sums will be made in connection with considerations of hatching. The use of thermal units is common in salmonoid culture for predicting hatching dates. Battle (2), Embody (3), and Price (13) have summarized much of the literature as it applies to development in salmonoids. Most of the literature indicates that the rule of thermal

sums does not hold over the whole range of temperatures to which various species have been subjected. The number of thermal units, calculated from 0° C, required to bring about hatching increases, directly, with temperature. The use of 0° C as zero for determining thermal sums has been questioned by Embody (3). The source of confusion is the lack of understanding of the implication of the rule of thermal sums. Properly, thermal sums should be based on the "physiological zero" of the organism for the particular processes under consideration, here developmental zero. Developmental zero can be calculated; in this instance it is the x-intercept of the line of the points for the rate of development, based on times to hatching, against developmental temperatures. For lake trout, zero for embryonic development is approximately $-1.5°$ C. Figure 4 shows the line for the present data; Embody's data are also plotted for comparison. His figures, which are based on hatchery records and not controlled experiments, fit the calculated line rather well. From the thermal unit values based on development zero and given in Table IV, it is questionable whether or not there is a significant trend in the high oxygen lots. Loss of the 5° C lot unfortunately poses a serious obstacle in this consideration. The figures for the lower oxygen levels suggest that the effects of low oxygen increases with temperature.

It cannot be supposed that successful development would occur at all temperatures between developmental zero, which is in general an extrapolated

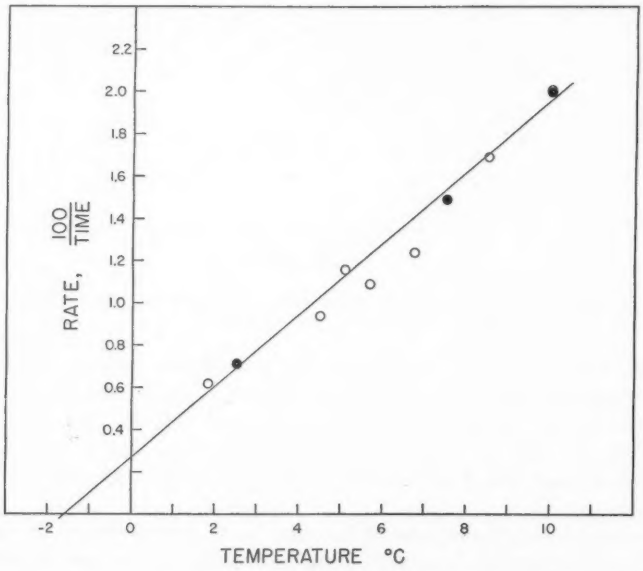

FIG. 4. Graphic determination of developmental zero for lake trout embryos by extrapolation of the line of fit of the points for the rates of development, based on times to hatching, against developmental temperatures. The developmental zero is approximately $-1.5°$ C. The open circles are Embody's data (3) taken from hatchery records.

point, and the upper lethal temperature. In all probability the lower lethal temperature is somewhat above developmental zero. Whitefish (*Coregonus clupeaformis* Mitchill) do not develop beyond the gastrula at 0° C (13).

Disturbances in the rate of differentiation can cause morphological variations ranging from an innocuous meristic change to the developmental failure of whole organs and large portions of the body. The former appear to result from relatively smaller changes in the developmental rate (Hubbs (10), Tåning (15)) whereas the latter are due to temporary arrests of some or all of the differentiation processes. If fish embryos are subjected to temporary severe stress in early gastrula stage (germ ring), twinning or head distortions, or both, are frequent consequences as demonstrated by Stockard (14). Similar stresses imposed during later axial differentiation can result in deformities of the trunk such as those reported by Alderdice *et al.* (1) for chum salmon and those found in lake trout.

There is evidence indicating that differentiation of successive structures in the salmonoid embryo is confined to rather rigid limits. If a structure is not formed, by reason of a metabolic block, in its normal position in the embryonal sequence, it will not develop at all. Chum salmon (1) and lake trout embryos subjected to severe stress failed in many instances to develop complete trunks. The chum salmon were removed from their hypoxial environment after 7 days, in time to develop, although rather imperfectly, a caudal fin on the terminal somite. The lake trout which failed to develop the posterior portion of the trunk were subjected to the stress (2.5 p.p.m. O_2, 7.5° C) for a much longer period and they did not develop caudal fins. However, in many cases other structures were not affected and their development proceeded normally and in their usual sequence albeit more slowly. All embryos do not respond equally to the same conditions of stress and some embryos developed normally and hatched successfully in the low oxygen levels at all temperatures except 10° C where all died before hatching. Egg size might account, at least in part, for the differential response. The surface to volume ratio would be less critical in smaller eggs and would permit relatively more oxygen to enter the egg.

In natural conditions it is possible for lake trout eggs to be subjected to lowered oxygen levels in lakes, as a result of winter oxygen depletion, lack of circulation, and silting. Minor reductions in dissolved oxygen levels would not seriously affect the developing embryos or bring about a high incidence of mortality. However, the data indicate that if oxygen levels fall below about 40% of air saturation at temperatures of 3° C or less, the embryos might well be subjected to stress during development. John and Hasler (11) state that the alevins of the cisco (*Leucichthys artedi* Leseuer) are handicapped by prolonged ice cover on lakes since this prevents illumination of the water and they are unable to see and, hence, to feed. This could apply to the lake trout as well. Moderately reduced oxygen levels which would delay hatching but not cause deformities or death would, in such a case, enhance the probability of higher survival in the alevins.

Acknowledgments

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ABNORMALITIES IN SOME IMMATURE ELATERIDAE (COLEOPTERA)[1]

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Abstract

Prothetely was observed in 13 elaterid larvae, and metathetely in numerous pupae, that were exposed to near-freezing temperatures several days before metamorphosis would have occurred normally. Prothetely also occurred in nine larvae, and metathetely in seven pupae, that were reared in soil that is not inhabited by the species in nature. In dry soil, many larvae molted abnormally and developed a minor deformity of the ninth abdominal segment; some larvae molted only partially and died.

Several embryos, 75 newly hatched larvae, and six larvae in later instars had aberrant ninth abdominal segments. In two other newly hatched larvae the third to ninth abdominal segments were missing. These aberrations appear to be external manifestations of a genetic disturbance that occurred in the parent generation. They were usually fatal in the embryonic or early larval stage, but a few of the newly hatched larvae and the six larger larvae, all of which exhibited nearly symmetrical aberrations of the ninth abdominal segment, developed and behaved normally in the larval stage.

Introduction

Five abnormalities[3] were observed among many thousands of specimens examined during studies on the biology and control of Elateridae at the Saskatoon laboratory. One affected the pupal, three the larval, and one the embryonic and larval stages. Some of the abnormalities are minor morphological aberrations that have caused some confusion in identification; others are lethal and, occasionally, of economic importance; all appeared under controlled or determinant conditions indicative of some of the causative factors. Except for prothetely, the following abnormalities are, as far as is known, new records for Elateridae.

Descriptions and Observations

Prothetely

Prothetely (premature formation of pupal characters in larvae) was observed in 3 larvae of the Puget Sound wireworm, *Ctenicera aeripennis aeripennis* (Kby.), 6 of the prairie grain wireworm, *C. a. destructor* (Brown), 1 of *Agriotes* sp., probably *limosus* Lec., and 12 of *Hypolithus bicolor* Esch. Most of the prothetelous characters of these larvae are similar to those of the prothetelous elaterid larvae described by Hyslop (3), Stone (5), Williams (8), Thomas (6), van Zwaluwenburg (11), Bianchi (2), and Becker (1). However, there is a greater variation among these larvae in the number and degree of development of the abnormal structures. They range from barely noticeable wing buds only, in some of the larvae, to several pupal structures, including a

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[3]Representative specimens were deposited in the Canadian National Collection of Insects.

ninth segment that is pupiform in structure but larviform in sclerotization, in the larva of *Agriotes* sp. (Fig. 1A). Five of the specimens have distinct pupiform female genital palpi on the posterior margin of the ninth abdominal segment (Fig. 1B), and seven others have a pair of small genital papillae on the posterolateral margins of the same sternum (Fig. 1C).

All these instances of prothetely occurred in apparently normal larvae that were reared or stored in the laboratory, and that were in or near the prepupal period when affected. The abnormality appeared in 6 of 120 specimens of *C. a. destructor*, in 2 of 80 of *C. a. aeripennis*, and in 1 of 5 of *Agriotes* sp. that were reared at a temperature near 68° F in moist soil, which they do not inhabit in nature. In 1 of 77 specimens of *C. a. aeripennis*, and 12 of 75 of *H. bicolor*, prothetely appeared when they molted, within 10 days at temperatures near 68° F after storage for about 4 months at 32° to 35° F.

The specimens of *H. bicolor* that molted or pupated later than 10 days after they were removed from storage developed normally. Some of the larvae molted one or more times while in the prothetelous condition, but there was no appreciable change in the abnormal structures after a molt. Ecdysis was abnormal in only one larva, which had exceptionally large wing buds. One specimen of *C. a. aeripennis* developed into an apparently normal female beetle. The others died, were fixed for histological sectioning, or were preserved.

Metathetely

Metathetely (imperfect metamorphosis of pupae) was first observed in 1952 in four pupae of *C. a. destructor* and three pupae of *C. a. aeripennis* that were reared in soil in individual containers at a temperature near 68° F. The appearance of the pupae and the duration of the pupal period seemed normal. At eclosion, however, each acquired a beetlelike head, thorax, and external genitalia, but the abdomen, wing pads, and usually the coloration remained pupiform (Fig. 2). Two of the pupae shed their exuviae normally; the others remained at least partially encased in the exuviae. This abnormality was fatal. Death usually occurred before the integument darkened and hardened.

In October, 1954, a similar abnormality was observed in seven living and numerous dead pupae of *C. a. destructor* in a field in fallow near Cochin, Sask., where it was estimated to affect more than 50% of the pupae. Ten apparently normal pupae were taken from the field at this time, and were held at 70° F on loam soil with a moisture content of 15%. Four pupae showed signs of ecdysis within 2 days after collection, when the abnormality appeared, and they died within another 2 days. The other six remained in the pupal stage for a week or more and developed into apparently normal adults.

Metathetely appeared in *C. a. destructor* in the Cochin field in a year when the precipitation during July, August, and September was approximately 1.0, 3.5, and 1.25 inches, respectively, above normal, and the mean temperatures, 1.7°, 3.7°, and 2.0° F, respectively, below normal. These

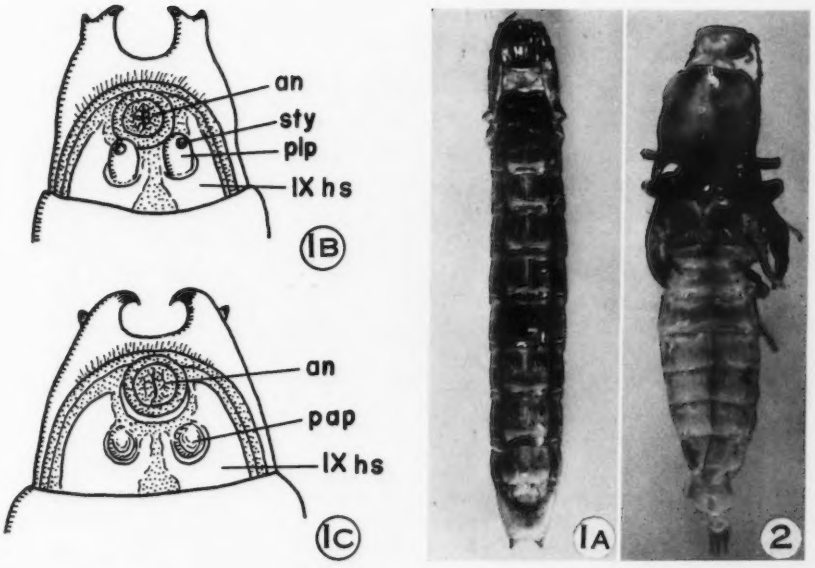


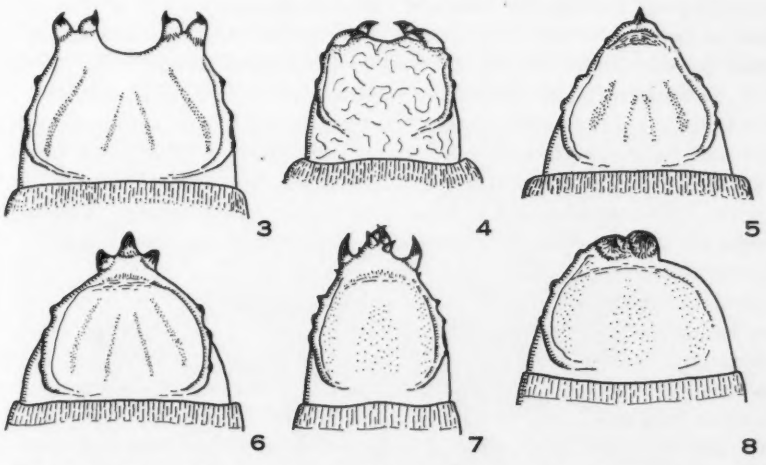
FIG. 1. Prothetelous larvae, ventral views. A, *Agriotes* sp., probably *limosus* Lec. B, ninth abdominal segment of *Hypolithus bicolor* Esch., showing genital palpi. C, ninth abdominal segment of *H. bicolor*, showing genital papillae. *an*, anus; IX*hs*, ninth hemisternite; *pap*, genital papilla; *plp*, genital palpus; *sty*, stylus.

FIG. 2. Metathetelous pupa of *Ctenicera aeripennis destructor* (Brown), dorsal view.

factors, in combination with the longer than normal periods of cloud cover, resulted in abnormally low soil temperatures during and after the normal pupation period. In the individuals that were reared from the larval stage, the abnormality appeared when the temperature was held continuously near 68° F. However, the soil that they were reared in during the larval and pupal stages had acquired an abnormal salt content from frequent additions of tap water. Also, the specimens of *C. a. aeripennis* were reared in black soil, which they do not inhabit in nature (10).

Abnormal Larval Ecdysis

Several instances of abnormal ecdysis in larvae of *C. a. destructor* were observed in the laboratory when the larvae molted in nearly air-dry soil. The old cuticle split normally along the dorsal mid-line and, in most of the affected larvae, was shed from all but the ninth abdominal segment. The new integument on the freed segments of the body expanded and hardened normally. The ninth segment, still encased in the old cuticle, expanded little and mostly dorsoventrally before the new integument hardened. This resulted in a deformity in the segment. It is usually wrinkled, and smaller and more strongly rounded (Fig. 4) than that of a normal larva (Fig. 3). The urogomphi are embedded in the posterior margin of the tergum and appear as four separate prongs, directed medially, rather than as the normal bifid pair, directed posteriorly. The caudal notch is considerably reduced. The exuviae were eventually lost during the movements of the larvae in the soil,



FIGS. 3–6. Ninth abdominal segments of larvae of *C. a. destructor*, dorsal views. Fig. 3. Normal. Fig. 4. Deformed through abnormal ecdysis. Fig. 5. Aberrant, with one terminal urogomphal prong. Fig. 6. Aberrant, with three terminal urogomphal prongs.

FIG. 7. Aberrant ninth abdominal segment of larva of *C. morula* (Lec.), with four urogomphal prongs and two additional protuberances, dorsal view.

FIG. 8. Aberrant, asymmetrical ninth abdominal segment of *C. a. destructor*, with two urogomphal prongs, dorsal view.

and the sooner this happened, the less severe was the deformity. Once the larvae were free of the exuviae, the deformity did not appear to affect their activity. However, a few of the affected larvae of this species, and all of several larvae of *H. bicolor* that were similarly abnormal, were unable to free the head and legs from the exuviae, and died.

Numerous larvae of *C. a. destructor* with a similar deformity of the ninth segment were taken in the field. The percentage of the larvae in the collections that were affected varied from none to approximately 35 in different years, periods within years, and fields. It was usually associated with dry and hot soil conditions, and the more extreme these conditions the higher was the percentage of the larvae affected. When larvae with this deformity were reared at 68° F in loam soil with a moisture content of 15%, the deformity disappeared at the next molt and subsequent development was normal.

Aberrations of Ninth Abdominal Segment in Larvae

Aberrations of the ninth abdominal segment were observed in six elaterid larvae taken in the field. The ninth abdominal segment of one larva of *C. a. destructor* tapers posteriorly to a single mediodorsal prong and a medioventral protuberance (Fig. 5). In another larva of this species (Fig. 6) and in one of *H. bicolor*, the ninth tergum tapers similarly but terminates in one median and two lateral prongs situated symmetrically on the tergum. Two larvae, one of *Athous nigripilis* Mots. and one of *C. morula* (Lec.), have four urogomphal prongs situated symmetrically on the tergum, but the two medial prongs are side by side and, on first glance, appear as one prong. Also the larva of *C. morula* (Fig. 7) has two additional protuberances on the dorsal surface of the tergum, anterior to the medial prongs. One larva of *C. a. destructor* (Fig. 8) has an asymmetrical ninth abdominal segment; one lateral margin of the tergum appears normal, the other is reduced in sclerotization and lacks the three heavily sclerotized "teeth". This larva has two urogomphal prongs situated asymmetrically on the terminal portion of the tergum. The caudal notch is missing in all the above larvae. Two of the larvae were reared through several molts without any apparent change in the aberrations.

Similar aberrations of the ninth abdominal segment were observed in the late embryonic and earliest larval stages of *C. a. destructor*. Of eggs laid in the laboratory in May and June, 1958, by 10 female beetles taken in the field earlier in the spring, over 200 were incubated in soil with a moisture content of about 15% at each of the temperatures 50°, 68°, and 86° F. Of 514 larvae that hatched, 439 were apparently normal and 75 had aberrations of the ninth abdominal segment. The remaining eggs failed to hatch, although most of them showed varying degrees of embryonic development. In 22 of these, the chorions had split but the larvae failed to emerge.

The degree of deformity in the aberrant embryos and newly hatched larvae varies from a much distorted ninth abdominal segment, in which the pos-

terior margin of the tergum is rounded and has no vestiges of urogomphi or of a caudal notch, to a nearly normal ninth abdominal segment that has a caudal notch and symmetrically situated, but reduced, urogomphi. In general, the reduction and distortion of the ninth segment were greatest in the embryos that died at a late stage of development and in the larvae that died shortly after eclosion, and were less in the few aberrant larvae that displayed normal activity after eclosion. Embryos that died at an early stage of development were not examined.

There was no significant difference in the percentage of the eggs that hatched, or of the larvae that were normal, among the three temperatures of incubation (10). The aberrations appeared only in larvae that hatched from eggs that were laid by three of the beetles. These three beetles also laid most of the eggs that failed to hatch, though a few of their eggs developed into apparently normal larvae. The larvae that hatched from the eggs of the other seven beetles appeared normal.

Half-monster Larva

Woodworth (9) described a newly hatched double monster larva of *Limonius canus* Lec., and Kring (4) described one of *L. agonus* (Say). In the former, the 7th to 10th abdominal segments are duplicated; in the latter, the 9th and 10th abdominal segments are duplicated.

In 1952, two half-monster larvae hatched from eggs laid in the laboratory by one female of *C. a. destructor*. In these larvae, a reduction, rather than a doubling, of body segments occurred. The head, thorax, and legs of each are similar to those of a normal newly hatched larva except that the thoracic segments are enlarged to approximately twice the normal widths. The first segment of the abdomen appears normal, but the posterior portion of the second and all the succeeding abdominal segments are missing (Fig. 9).

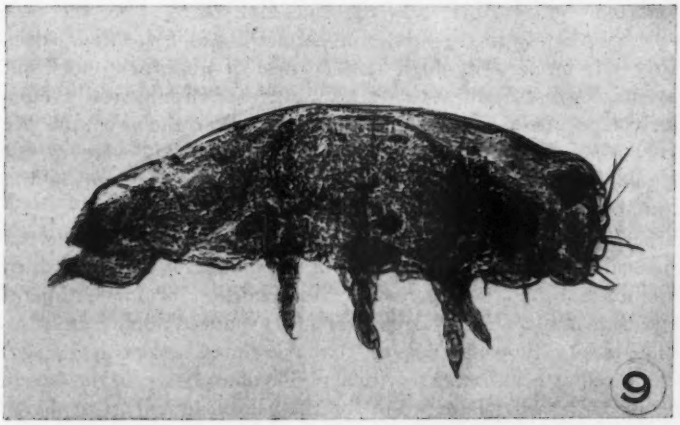


FIG. 9. Photomicrograph of newly hatched half-monster larva of *C. a. destructor*, lateral view.

The truncate posterior wall of the second abdominal segment is covered by a cuticula that is folded into several protuberances and bears several hairs. There is no visible anal orifice. Both larvae were abnormally inactive. They were alive 4 days after they were hatched, when one was preserved in toto on a microscope slide, and the other was sectioned for examination of the internal anatomy. Except for a much enlarged digestive tube, the internal organs of the latter appear similar to those of a normal larva at the same stage of development, but they terminate abruptly in the second abdominal segment.

The half-monster larvae hatched with 170 normal ones from a group of 177 eggs laid by one female. The five eggs that failed to hatch were attacked and apparently killed by mold. These were the only abnormal larvae among more than 10,000 that hatched from eggs that were handled and incubated similarly during that year.

Discussion

Wigglesworth (7) indicates that prothetely and metathetely are conditions resulting from some upset in the balance of hormones during development, and are induced at times by abnormal environmental conditions. He suggests that, in molting *Rhodnius* larvae, low temperature upsets the hormone balance in favor of the juvenile hormone and causes metathetely; high temperature upsets the balance in favor of the molting hormone and causes prothetely.

Nearly all the instances of prothetely in elaterids reported here and previously appeared in larvae that were reared or stored out of their natural habitats. This suggests that the causative factors were environmental. Thomas (6) concludes that high temperature and abnormal moisture were probably not the factors that induced this abnormality. Stone (5) considers that factors other than temperature, moisture, and food were causative. Hyslop (3) considers that abnormal humidity at or shortly before pupation, rather than abnormal temperature, was the causative factor. At the Saskatoon laboratory, prothetely in the larvae of *Ctenicera* and *Hypolithus* species was apparently not induced by high temperature or abnormal moisture. The observations suggest that at least two other environmental factors were concerned: exposure of larvae that were in or near the prepupal period to near-freezing temperatures, and rearing of larvae that were in the later instars in soil that had acquired an abnormal chemical content, or that they do not inhabit in nature.

Metathetely was observed in *C. a. destructor* in the field only in one year, when low soil temperatures and excessive soil moistures prevailed during and after the normal pupation period. The condition of low temperature is similar to that under which Wigglesworth (7) induced metathetely in *Rhodnius*. The observations also suggest that there may be a critical stage within the week preceding eclosion, or a critical period of exposure, in the development of pupae in relation to temperature for the establishment of a physiological balance that favors eclosion. In the laboratory, metathetely appeared in the pupae of *C. a. aeripennis* and *C. a. destructor* that were reared from larvae

in moist soil at a temperature continuously near 68° F. Thus, low temperature did not induce the abnormality in these specimens. However, they were reared during the larval and pupal stages in soil that they do not inhabit in nature, or in soil that had become abnormally saline from the frequent additions of tap water, and the only apparent causative factor was an abnormal chemical content of the soil.

Although prothetely is apparently not of economic importance in the field, some chemical constitution of the soil appears to induce it in laboratory rearing and, consequently, also may be a factor that limits the distribution of the species in nature. Metathetely drastically reduced adult populations of *C. a. destructor* in the field, but the presence of the causative factors at the critical stage of development is infrequent in the Prairie Provinces.

The apparent causative factors for prothetely and metathetely in the instances reported here were similar, and these factors appeared similarly to induce the two abnormalities just before metamorphosis. These observations suggest that in these elaterids the two abnormalities are manifestations of a similar physiological disturbance that causes incomplete metamorphosis, and that the different external manifestations are a product only of the stage of development affected.

The abnormal ecdysis of the larvae was apparently caused primarily by a lack of adequate soil moisture immediately before and during the molting period. Although fatalities from this abnormality were observed in rearing and in nature, it does not appear to be a serious threat to field populations and can easily be controlled in laboratory studies.

The aberrations of the ninth abdominal segment that were observed in the six elaterid larvae in the field appear to be similar to those observed in the embryos and newly hatched larvae in the laboratory. The outstanding characteristic of five of these larvae is the symmetry of the aberrant structures, which the laboratory observations indicated to be a manifestation of the less severe form of the abnormality. This abnormality, and that of the half-monster larvae, appears to be the result of some genetic disturbance in the parent generation. Although the abnormalities occurred rarely, they were fatal to most of the affected individuals in an early stage of development. As individuals in these early stages of development are not normally found in the field, the frequency of occurrence and the effect on populations of these abnormalities in nature are difficult to assess.

Abnormalities such as those described above are obviously indicators of some disturbances in the physiological or the genetic processes. They show promise as tools for elucidating the processes of growth and development, the origins of structures, and the factors that limit the distribution of species.

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A SIMPLE METHOD FOR REARING PURE POPULATIONS OF THE FOLIAR NEMATODE, APHELENCHOIDES RITZEMABOSI, IN THE LABORATORY[1]

K. C. SANWAL[2]

Abstract

A direct infestation method as a means of rearing pure populations of *Aphelenchoides ritzemabosi* is described. A mature female is placed directly on the ventral surface of the leaf on a tiny droplet or a thin film of water. Reproduction takes place after successful entry of the nematode into the leaf tissue. The progeny of this female is then transferred to new leaves and the procedure is continued. Methods of maintaining high humidity around the leaves are described. The use of 'mist propagation frames' to enhance the spread of disease in greenhouse beds is recommended.

The significance of rearing pure populations of nematodes from a single female need not be emphasized. A readily available stock of pure population of any nematode species is essential for critical morphological studies to elucidate the variations in a single species and to establish sound morphological characters for taxonomic purposes. It is possible to rear many free-living and a few plant-parasitic species of nematodes in agar media in which a suitable fungus is growing. Thus Christie and Crossman (1) have successfully cultivated *Aphelenchoides fragariae* in agar plates inoculated with a fungus. *Ditylenchus destructor*, the potato-rot nema, has been successfully cultivated on agar-fungus plates in this laboratory for the first time. Recently Todd and Atkins (3) have described a method for culturing mass populations of *A. bessyi* from infested rice seeds. Culture of *A. ritzemabosi* on agar plates in association with a fungus, attempted by Christie and Crossman (1) and the author, have not been successful. By the following simple method of direct infestation, however, it is possible to rear large populations of *A. ritzemabosi* from a single female in a very short period and also in the natural habitat of the parasite.

All glassware, enamelware, pots, and sand used in the experiment are thoroughly sterilized. A healthy and clean stock of chrysanthemum and aster plants is maintained in the greenhouse. These plants are preferably grown from seeds in sterilized soil. Plants grown from uninfested cuttings, however, may be used but it is important to check and recheck different parts of plants and soil to ensure that the plants are free from any infestation. The initial experiments in this laboratory have been conducted with plants grown from seeds, and later on with plants grown from healthy and uninfested cuttings.

A large number of worms is extracted from an infested chrysanthemum leaf by the Baermann funnel technique. These worms form the mixed stock

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population from which pure lines are to be established. A small 20-ml beaker (Fig. 1, *a*) is filled up with sand which is watered till completely wet. The beaker is then placed in a 15-mm petri dish (*p*) filled with clean water.

A young and healthy leaf is taken from the clean uninfested stock of chrysanthemum that is being maintained in the greenhouse. The leaf is moistened by being dipped into clean water in a beaker. The excess of water adhering to the surfaces of the leaf is shaken off with a vigorous jerk. This leaves tiny droplets and thin films of water still adhering to the surfaces of the leaf. A single female is then picked up on a bamboo splinter from the mixed stock population previously collected from the infested leaf and is transferred to one of the tiny droplets or thin film of water adhering to the ventral surface of the leaf. This transfer is checked under the dissecting microscope as the worm is easily visible in the droplet of water on the leaf. The leaf is now stuck into the small beaker filled with wet sand (*a*) as described above. Both the petri dish containing water (*p*) and the beaker containing

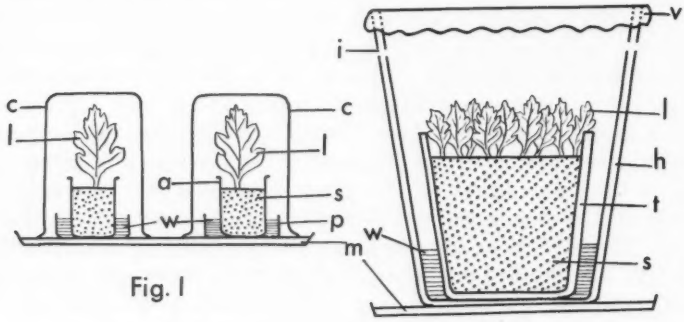

Fig. 1

Fig. 2

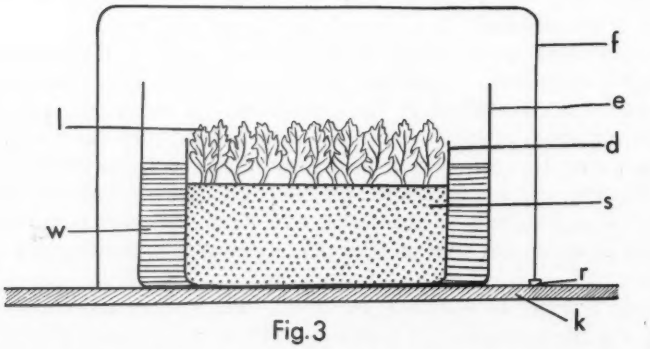

Fig. 3

FIGS. 1–3. Apparatus for rearing pure populations of *Aphelenchoides ritzemabosi*.
ABBREVIATIONS: *a*, 20-ml beaker; *c*, 250-ml beaker; *d*, 100-mm crystallizing dish; *e*, 150-mm crystallizing dish; *f*, 190-mm crystallizing dish; *h*, 8-in. earthen pot; *i*, hole for ventilation; *k*, top of laboratory table; *l*, leaf; *m*, enamel dish; *p*, 15-mm petri dish; r, cork foot; *s*, sand; *t*, 5-in. earthen pot; *v*, plastic cloth cover; *w*, water.

the leaf (*a*) are covered by inverting a 250-mm beaker (*c*) over them. Following the same procedure, 12 leaves are planted in 12 beakers. All these beakers are set in a clean, sterilized enamel dish and kept at room temperature either in the laboratory or in the greenhouse, protected from direct sunlight. The small spout on the edge of the inverted beaker keeps the system well ventilated and the water in the petri dish maintains a high humidity of the air around the leaf. This is further supplemented by spraying the surfaces of the leaf and the inner surface of the inverted beaker with a fine atomizer every second or third day. The leaves should not be kept wet for long periods, however, as this stimulates fungus infection and rotting of the leaves. If desired, another identical set of 12 beakers with 12 approximately similar leaves from the same stock of plants but without nematodes can be assembled to serve as control. All the beakers are marked for subsequent record.

Symptoms of infection make their first appearance within 7–11 days as brownish blotched areas on the leaf. These areas eventually turn dark brown and dull black and are limited by the veins (17–21 days). If the infection is allowed to proceed, the whole leaf is gradually infested. Out of the 12 leaves planted with one female on each, establishment of the nematode is usually obtained in at least 3–4 leaves, although as many as 9 out of 12 attempts may be successful. The appearance of dark areas on the leaf does not always mean that the leaf is infested, as this may be due to fungus infection or decay. Later the leaves are teased out in water and checked for nematode infestation. The leaves in the control beakers may also indicate disease symptoms identical with the leaves which were experimentally infested, but they have never been found harboring nematodes.

An infested leaf is teased out in water and the worms are extracted by the Baermann funnel technique. The number of worms recovered depends on the time interval. (After 22 days, one chrysanthemum leaf yielded 34 worms. An aster leaf yielded approximately 150 worms 37 days after inoculation. Infestation proceeds faster in aster leaves than in those of chrysanthemum but the leaves of the former seem to be more prone to fungus disease than the latter. For an initial experiment, it is easiest to start with chrysanthemum leaves. Aster leaves may be used in the following part of the experiment.) These populations that are the progeny of a single female may be now distributed in larger numbers (5–10 on each leaf) with the same procedure as in the initial experiment (one female on each leaf). Instead of isolating each leaf in a single beaker these leaves are planted together in a 100-mm crystallizing dish filled with wet sand (Fig. 3, *d*). This dish is immersed in a 150-mm dish (*e*) filled with clean water. Both these dishes are covered by inverting a 190-mm crystallizing dish (*f*) over the top. A small cork foot (*r*) applied to the edge of the inverted dish provides for ventilation. The air around the leaves is kept moist by the water in the surrounding dish and by occasionally spraying the leaves and the inside of the inverted dish with an atomizer. This spraying, however, should be done only after the tiny droplets and thin film of water in which the nematodes have been transferred have dried (24 hours or more) to ensure that the nematodes have enough time to enter the

leaf tissue and are not washed down with the water spray. This also applies to spraying in the first experiment with one leaf in one beaker.

It has been found that the disease is established in the leaf even if the nematodes are transferred to the dorsal surface of the leaf. This operation is in fact easier than placing the nematodes in the tiny droplets of water on the ventral surface of the leaf, because the droplets of water tend to roll off the leaf on account of the smooth waxy texture of the ventral surface. Drops of water containing 5–10 nematodes can be transferred to the dorsal surface of a wet leaf with a pipette without danger of the water rolling off the leaf. The texture of the leaf on the dorsal surface tends to absorb and distribute the water drop evenly on the surface.

Within a period of 5–9 days the symptoms of invasion make their first appearance, the visibly affected area gradually spreads, and within 23–45 days all the leaves have become completely infested. Each leaf yields hundreds of nematodes. These worms are transferred again in greater numbers (20–30 on each leaf) to new aster or chrysanthemum leaves, with the same procedure as above. The larger the number of nematodes placed on a leaf, the faster the disease spreads and in a very short time it is possible to have a large number of infested leaves, each leaf yielding thousands of worms. Other pure populations can be reared by propagating the progeny from each infested leaf from an initial inoculation (i.e., of one nematode on one leaf).

An alternative method, to replace the second part of the experiment (more than one nematode on each leaf), is as follows: After transferring the nematodes onto the leaves, the leaves are planted in wet sand in a 5-in. pot (Fig. 2, *t*) the drainage hole of which has been sealed with a rubber stopper and wax. This pot, carrying the leaves, is put inside another 8-in. pot (*h*) without a hole at the bottom. Two small holes (*i*) are drilled towards the upper margin of the outer pot. The outer pot is filled with water to a height shown in the figure, and is covered on top by a banded, translucent plastic cover (*v*). The water in the outer pot, by keeping the walls of the pot moist, maintains a high humidity around the leaves and the two holes on the walls of the pot provide for ventilation. This method is excellent for maintaining a high humidity around the leaves, but the water level in the outer pot has to be regulated from time to time. The water should be just enough to keep the walls of the pot in a moist condition but not thoroughly drenched and soaked, as excess of moisture results in rotting and decay of leaves and also induces fungus growth.

If desired, the nematodes can be transferred to plants growing in greenhouse beds by transferring drops of water containing nematodes onto the surfaces of wet leaves. It has been found, however, that, under ordinary greenhouse conditions, the healthy plants are not easily infested, and, if they are, watering alone does not accentuate the rapid spread of the disease. The use of 'mist propagation frames' (2) has proved very effective in bringing about a rapid spreading of disease to the upper regions of the plant. The frame involves the use of a superfine sprayer which is automatically operated to spray a fine mist

of water on and around the plants for a few seconds at preset intervals. The plants and the sprayer are covered over by a plastic cloth tent which keeps the humidity inside the tent at a high percentage.

Acknowledgments

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NUTRITION OF THE PALE WESTERN CUTWORM, AGROTIS ORTHOGONIA MORR. (LEPIDOPTERA: NOCTUIDAE)

II. DRY MATTER AND NITROGEN ECONOMY OF LARVAE FED ON SPROUTS OF A HARD RED SPRING AND A DURUM WHEAT[1]

R. KASTING[2] AND A. J. MCGINNIS[3]

Abstract

Dry matter and nitrogen balances, determined for individual instars of the pale western cutworm fed ad libitum on wheat sprouts, showed that reliable data were obtained for only the fourth, fifth, and sixth instars. During the larval period a single cutworm consumed about 10 g of fresh wheat sprouts containing about 25 mg of nitrogen. Of the consumed food, 40–50% of the dry matter and 60–70% of the nitrogen were excreted. Five days after the fifth molt the fresh weights of larvae fed on sprouts of durum wheat were significantly less than those fed on sprouts of the hard red spring wheat, but the dry weights were the same. The quantities of nitrogen found in excreta and body tissue approximated that consumed by the larvae. In contrast, however, much of the consumed dry matter (14–33%) could not be accounted for; recovery varied with variety. The percentage of the dry matter utilized, about 50%, remained constant during the last three instars. Nitrogen utilization by fourth-, fifth-, and sixth-instar larvae ranged between 25 and 45%. However, a consistently lower percentage of the nitrogen was utilized from Thatcher than from Golden Ball sprouts. On both varieties the lowest values were obtained in the sixth instar. In the excreta the percentages of insoluble dry matter, insoluble nitrogen, and volatile bases showed no consistent change during the last four instars. Seventy to eighty per cent of the excreted nitrogen was soluble in 80% ethanol, and about one-fifth of this was in the form of volatile bases. The variety of wheat sprouts fed had little, if any, effect on the composition of the excreta.

Introduction

Balance measurements have contributed much to the knowledge of comparative values of foods in mammalian nutrition (9). Similar studies with insects are scarce (8). The present investigation was undertaken to determine if reliable balance measurements could be obtained for a plant-feeding insect. The pale western cutworm, *Agrotis orthogonia* Morr., was used because it is a large, fast-growing, phytophagous insect that can be reared in the laboratory (10). Comparative data are presented on dry matter and nitrogen balances for the cutworm when fed on sprouts of a hard red spring (*Triticum aestivum* L. emend Thell.) and a durum (*T. durum* Desf.) wheat.

Materials and Methods

Newly hatched larvae of the pale western cutworm, *A. orthogonia* Morr., were fed in groups of about 10 for the first two instars and thereafter individually in 60×15 mm petri dishes. The larvae were fed ad libitum on fresh sprouts of either Thatcher, *T. aestivum* L., or Golden Ball, *T. durum* Desf.,

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grown under standard conditions (10). Weighed quantities of sprouts were placed in each dish daily. Duplicate samples of the sprouts and the remnants in each dish from the previous day were analyzed for dry matter by being dried at 60° C *in vacuo* for 5 hours and for nitrogen by the micro-Kjeldahl procedure (1).

The larvae were weighed daily in groups of about 10 for the first and second instars and individually thereafter. They were removed from test 5 days after the fifth molt because our earlier work showed that larvae of this species reached their maximum weight about 8 days after the fifth molt and then rapidly lost weight, mostly in the form of moisture. The insects were sampled and analyzed for dry matter and nitrogen by methods described earlier (10).

Except for the first two instars, excreta from a single insect were collected on an instar basis by being washed daily from the petri dish with a small quantity of 80% ethanol (17). For the first two instars the excreta were similarly collected but on a group basis. All collections were stored at 2° C until analyzed. The excreta were separated into soluble and insoluble fractions by filtration through Whatman No. 1 filter paper. The insoluble fraction, which included the exuvium, was dried for 5 hours at 60° C *in vacuo* and weighed. Dry matter contents of the soluble fractions were determined from the weights of residues remaining after aliquots were dried on a steam bath and finally *in vacuo* at 80° C for 5 hours. The nitrogen contents of the insoluble fractions were obtained by analyzing the combined filter papers and residues and correcting for the nitrogen in the paper. Duplicate aliquots of the soluble fraction were also analyzed for nitrogen. The micro-Kjeldahl method was used for the nitrogen analyses. Total volatile bases, which presumably included nitrogen in the form of ammonia, monomethylamine, dimethylamine, and trimethylamine (13), were determined on aliquots of the soluble fractions by making them alkaline with NaOH, distilling them, and titrating them as in the micro-Kjeldahl procedure.

The soluble excreta from several fifth-instar larvae were analyzed chromatographically for urea, allantoin, and allantoic acid (5), and uric acid (11). Soluble amino acids were tentatively identified on 2-dimensional chromatograms prepared by the method of Porter *et al.* (12) except that the proportions of the *n*-butanol, acetic acid, water solvent were 4:1:5.

Results and Discussion

The mean values for the daily consumption of dry matter of Thatcher wheat sprouts by the pale western cutworm indicate variations within an instar (Table I). Dry matter consumption showed similar fluctuations for each of the instars. Further, other data showed that similar patterns of consumption occurred for the dry matter of Golden Ball wheat sprouts. The differences in consumption between the first 2 days of each stadium were expected because the molt could have occurred at any time during the 24 hours prior to observation and, therefore, the feeding period was necessarily reduced. Likewise consumption was expected to vary on the second and third days after the molt

when some of the insects entered the premolt condition. Dry matter consumption has been calculated for other insects (6, 7) by multiplying the consumption on a single day by the number of days in the stadium. This calculation was not reliable for the pale western cutworm because the quantities of sprouts consumed varied daily and the stadia varied in length (10). Consequently for balance studies with this insect it was necessary to measure the daily consumption directly.

TABLE I

Mean daily consumption of dry matter (mg) by larvae of the pale western cutworm fed on sprouts of Thatcher wheat

Instar	Days after molt			
	0	1	2	3
3	3.2±0.6*	4.8±1.1	1.6±0.7	5.6± 2.4
4	11.9±2.2	17.9±2.6	11.9±3.8	—
5	23.7±5.7	36.7±1.2	42.9±6.0	45.3± 5.9
6	54.4±6.9	84.0±9.0	78.3±4.4	65.3±17.0

*Standard deviation.

The average fresh weights on the day of each molt and 5 days after the fifth molt of larvae fed on sprouts of the two wheat varieties were:

Molt	Thatcher	Golden Ball
1	1.3	1.7
2	7.5	7.3
3	36	37
4	115	115
5	435	399
5+5 days	819	708

Although the larvae fed on Thatcher were significantly heavier ($P<.01$) at the end of the feeding period than those fed on Golden Ball, the dry weights of the two groups did not differ.

Table II compares the mean values for consumption, excretion, and retention as body tissue, of dry matter and nitrogen according to instar when larvae were fed either Thatcher or Golden Ball sprouts. The percentages of the consumed nitrogen recovered as excreta and body increase are also included as a measure of the reliability of the method. Assuming that there was negligible fixation of atmospheric nitrogen (15) or loss of gaseous nitrogen, then the nitrogen consumed by the insect should be recovered as nitrogen in the excreta and the body. Except for the third-instar larvae fed on Thatcher sprouts, the recoveries were not satisfactory for the first three instars. Nonetheless, data on these stages are presented because they give the relative magnitude of consumption and excretion. In general, the recoveries of nitrogen were good for the last three instars. Thus, at least for the last three instars of the pale western cutworm, balance studies are possible. The techniques that were developed for dry matter and nitrogen balance can be used for other dietary components such as amino acids. This was done with the silkworm by Stamm *et al.* (16).

TABLE II

Balance measurements for larvae of the pale western cutworm fed ad libitum on wheat sprouts

Instar	Variety	Dry matter (mg)			Nitrogen (mg)			% nitrogen recovery
		Consumption	Excretion	Retention	Consumption	Excretion	Retention	
1	Thatcher	1.05	0.83	0.10	0.05	0.03	0.01	80
	Golden Ball	0.78	0.66	0.16	0.03	0.03	0.01	130
2	Thatcher	2.07	1.52	0.67	0.11	0.06	0.13	170
	Golden Ball	2.53	1.69	0.69	0.13	0.04	0.11	115
3	Thatcher	11.3	6.1	4.01	0.61	0.26	0.33	96
	Golden Ball	16.6	5.7	3.70	0.82	0.25	0.32	70
4	Thatcher	43.2	23.2	12.3	2.38	1.42	1.10	106
	Golden Ball	48.4	21.3	13.7	2.20	1.21	1.03	102
5	Thatcher	157.2	81.9	53.4	8.77	5.75	4.28	114
	Golden Ball	174.4	77.5	53.0	7.99	4.41	4.11	107
6	Thatcher	281.9	136.4	68.8	14.12	10.58	3.26	98
	Golden Ball	287.2	130.1	63.2	12.71	8.76	2.92	92
Total	Thatcher	496.7	250.0	138.5	25.88	18.00	9.11	–
	Golden Ball	529.9	237.0	133.6	23.73	14.63	8.50	–

Eighty to ninety per cent of the total food consumption occurred during the fifth and sixth instars and only 0.1–0.2% in the first and second instars (Table II). The excretion of dry matter and nitrogen showed a similar distribution. This demonstrates again (10) the bias introduced when data are collected over the whole growth period without reference to stage of development.

For the last four instars the percentage of insoluble dry matter, insoluble nitrogen, and volatile bases did not change consistently with age (Table III). Of the excreted nitrogen, 70–80% was soluble in 80% ethanol and about one-fifth of this was in the form of volatile bases. The quantities of excreta for the first two instars were too small to obtain reliable values.

TABLE III

Analyses of excreta of pale western cutworm larvae fed on sprouts of wheat

Instar	Variety	Insoluble dry matter (%)	Insoluble nitrogen (%)	Volatile bases* (%)
3	Thatcher	52.5	22.5	18.0
	Golden Ball	56.1	18.7	13.2
4	Thatcher	58.6	19.7	14.8
	Golden Ball	54.9	19.8	9.6
5	Thatcher	58.1	16.8	16.7
	Golden Ball	54.5	20.0	14.2
6	Thatcher	59.7	25.9	21.6
	Golden Ball	59.8	29.8	20.0

*Percentage of total soluble nitrogen.

The soluble excreta of fifth-instar larvae contained uric acid, urea, allantoin, and at least 10 amino acids tentatively identified as the leucines, valine, alanine, glutamic acid, serine, glycine, aspartic acid, asparagine, threonine, and x-amino butyric acid. The soluble excreta from larvae fed on either Golden Ball or Thatcher sprouts appeared to be qualitatively the same.

Table IV shows that the percentage utilization of dry matter of fourth-, fifth-, and sixth-instar larvae was generally similar within a variety ($P > .05$). However, Golden Ball was utilized to a greater extent than Thatcher ($P < .01$). The dry matter conversion efficiency was lower ($P < .01$) in the sixth instar but the importance of this is not apparent. The percentage of the dry matter consumed that was recovered as excreted dry matter and increase in body weight was lower ($P < .01$) for larvae fed Golden Ball than for those fed Thatcher sprouts. This value did not vary consistently with stage of development even though it was lower ($P < .01$) in the sixth instar. In balance studies (14) with grasshoppers, there have been recorded larger dry matter losses that varied depending upon the plant species fed. Part of the unrecovered dry matter undoubtedly was oxidized to supply energy. Conversion of carbohydrates and proteins of food to fat in the insect would also cause a loss in dry matter.

TABLE IV

Utilization and conversion of dry matter and nitrogen by, and recovery of dry matter from, larvae of the pale western cutworm fed ad libitum on wheat sprouts

		Dry matter			Nitrogen		
						Efficiency of utilization‡	
Instar	Variety	Conversion efficiency*	% utilization†	% recovery	% utilization	Fresh wt.	Dry wt.
4	Thatcher	62	46	82	41	35	5.2
	Golden Ball	52	56	72	45	36	6.2
5	Thatcher	71	48	86	34	33	6.1
	Golden Ball	55	56	75	45	32	6.6
6	Thatcher	47	52	73	25	19	4.9
	Golden Ball	40	55	67	31	19	5.0

* $\frac{\text{Body weight increase (mg)} \times 100}{\text{Consumption (mg)} - \text{excretion (mg)}}$.

† $\frac{\text{Consumption (mg)} - \text{excretion (mg)} \times 100}{\text{Consumption (mg)}}$.

‡ $\frac{\text{Body weight increase (mg)}}{\text{Nitrogen consumption}}$.

The percentage utilization of nitrogen from Thatcher was consistently lower ($P<.01$) than that of Golden Ball sprouts (Table IV). For both varieties it was lowest in the sixth instar ($P<.01$). With one exception the values were in the 29 to 56% range reported for late instars of five other species of Lepidoptera (2, 3). Table IV also shows that fresh weight gains per unit of nitrogen consumed were lower ($P<.01$) in the sixth instar; on a dry matter basis the values for the different instars were the same ($P>.05$). The efficiencies with which the nitrogen of the sprouts of the two varieties was utilized by the larvae did not differ ($P>.05$).

Growth and development of the pale western cutworm are affected by the variety of wheat sprouts fed (4). The present results show that the larvae fed Thatcher sprouts grew larger than those fed Golden Ball sprouts but that the dry weights of the larvae and the total dry matter consumption were the same ($P>.05$). Although the larvae that were fed Thatcher sprouts consumed and excreted more nitrogen than did those fed Golden Ball sprouts, the efficiencies of utilization were not different. The differences in fresh weight of the larvae reared on the two varieties appeared to result from the different moisture content of the sprouts (10). An estimate of the moisture consumption showed that the larvae fed Thatcher sprouts consumed more than enough extra moisture to account for the difference in weight.

Conclusions

1. It was possible to measure the consumption, excretion, and increase in body tissue of dry matter and nitrogen for the fourth, fifth, and sixth instars of the pale western cutworm. For these stages it appears that the techniques developed can also be used for other dietary components such as individual amino acids.

2. The consumption of sprouts by cutworms varied from day to day within an instar. Consequently the most reliable method of determining total consumption was to measure consumption on successive days and obtain the cumulative total. Consumption and excretion values bulked over the whole growing period masked the results of the early stages because over 80% of the feeding occurred in the last two instars.

3. The balance data for the dry matter and nitrogen showed some differences between the wheat varieties. The difference in the final fresh weight of the larvae fed on the two wheat varieties cannot be accounted for by any factor measured but appeared to be associated with moisture consumption.

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ACCELERATION OF DEVELOPMENT OF LARVAE OF THE ONION MAGGOT, HYLEMYA ANTIQUA (MEIG.), BY MICROORGANISMS[1]

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Abstract

On a chemical diet, larvae of *H. antiqua* developed more rapidly in the presence of various living bacteria, of certain nonviable bacterial cells, or of the dialyzate of these cells. The stimulation by the nonviable cells was not affected when the cells were disrupted by sonic treatment. Additions of ashed cells to the chemical diet had no stimulatory effect. Some stimulation in rate of development was noted on addition of casein to the diets but to a lesser degree than that caused by equivalent concentrations of nonviable cells. The growth rate and the rate of metamorphosis stayed in phase with one another when development was accelerated.

Introduction

The nutrition of larvae of the onion maggot, *Hylemya antiqua* (Meig.), has been studied at Ottawa since 1953 with chemical diets and axenic rearing techniques. Larvae grown on the best chemical diet require 6.3 to 7 days from hatching to reach the third instar. Eighty per cent of the larvae reared in this controlled diet become adults, some of which lay viable eggs (3).

It was observed that contamination of the chemical diets by microorganisms frequently accelerated development of the larvae both on the control diet and on incomplete diets that support subnormal development when fed under axenic conditions (4).

This paper describes a series of experiments on whether one or more specific development-accelerating factors are present in bacterial cells.

General Methods

The assay methods were similar to those used previously (2, 3). Larvae were kept individually in 1-dram screw-cap vials in an incubator at 25° ± 0.5° C throughout the assay period and were inspected daily. The control diet was the low level amino acid diet described previously (3). The statistical comparisons were made by using Duncan's multiple range and multiple *F* test (1).

Experimental

Addition of Various Bacteria as Companion Organisms

Attempts were made to determine whether any species of microorganism gave greater stimulation of the development rate than *Bacillus circulans*, which shortened the time required for larvae to reach the third instar by 2 to

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2.7 days (2). Nine species of bacteria were inoculated into diet tubes at the time of egg transplantation. These organisms consisted of type cultures of *B. circulans* and *Escherichia coli*, isolates of *B. circulans* from the eggs and of *B. cereus* from the gut of the onion maggot, and five unidentified chance contaminants of diet tubes. All bacteria grown as companion organisms shortened the time needed for the larvae to reach the third instar by approximately 2 days. None of the bacteria tested accelerated the development more than *B. circulans*. *E. coli* stimulated development until the third instar but the average time needed from hatching to pupation was 13.4 days compared with 13.1 days for the control diet. With the other bacteria the time needed from hatching to pupation was about 3 days less than on the control.

Addition of Dead Bacterial Cells

Previous studies (2) showed that addition of nonviable *B. circulans* cells to diets sterilized by filtration significantly stimulated larval development. Since filtration removes a portion of the less-soluble diet constituents, it was possible that bacterial supplements merely substituted for these nutrient deficiencies. To study the effect of nonviable cells in diets not modified by filtration, spores and vegetative cells of *B. circulans* were added to an unfiltered diet in a series of experiments.

B. circulans cells were grown on Penassay base agar[5] for 5 days at 37° C. After incubation, the cells were removed, centrifuged at 4500 r.p.m., resuspended in distilled water, and centrifuged. The washing and centrifugation were repeated 3 times. After the final centrifugation, spores and vegetative cells were separated and each was resuspended in distilled water.

Cell and spore suspensions were prepared so that the final concentration per 0.3 ml was equal to 8.1 mg (dry weight). Tubes containing 1.5 ml of control diet were melted and held at 50° C while 0.3 ml of spores or vegetative cells was added. The supplemented diet tubes were autoclaved at 15 lb pressure for 30 minutes, shaken to ensure uniform turbidity, and immediately chilled.

Larvae developing on the diets supplemented with spores and vegetative cells reached the third instar in an average of 4.8 and 4.6 days respectively as compared with 6.8 days for the control diet, the differences being significant statistically. Evidently the stimulatory factor is an intrinsic, heat-stable part of the nonviable cell.

To secure quantitative data concerning the development accelerating potency of bacterial cells, a dosage-effect test was conducted in which the diet was supplemented with various concentrations of dead cells of *B. cereus*. The cells were grown in a glucose – yeast extract agar for 3 days at 26° C, harvested, washed, and freeze-dried. Known weights of cells were resuspended in distilled water and added to the diet tubes as previously described.

The larvae developed progressively faster as the level of the *B. cereus* supplement was raised (Table I). As little as 0.5 mg of cells per milliliter of diet brought about a significant acceleration. Levels as high as 6 mg per milliliter of diet were needed to give an effect approximating that shown

[5]Difco Laboratories Inc., Detroit 1, Mich.

when living bacteria were included as companion organisms. The time required from hatching to pupation showed a parallel dosage effect, maximum acceleration occurring at the highest level of supplement, when the development time was 6.1 days less than that of larvae on the axenic control diet.

TABLE I

Development of *H. antiqua* larvae on a chemical diet supplemented with various concentrations of nonviable *B. cereus* cells

Nonviable *B. cereus* cells, mg per ml of diet	Number tested	Days from hatching to reach			
		Third instar		Pupa	
		Av.	Range	Av.	Range
None (axenic control)	19	7.1	6–9	16.3	13–21
None (monoxenic control)†	24	4.4	4–5	10.1	9–11
0.25	12	7.1	6–8	14.4	12–16
0.5	14	6.6	6–8	14.0	12–16
1.0	21	6.2*	6–7	13.0	11–17
2.0	20	5.9*	5–7	12.3	11–14
3.0	13	5.8*	5–7	11.8	10–14
4.0	17	5.8*	5–7	11.3	10–13
6.0	19	5.1*	5–6	10.9	9–11
8.0	18	5.1*	4–6	10.2	9–12

*Significantly different from axenic control; necessary difference, $P = 0.01 = 0.68$ days.
†Inoculated with living *B. cereus*.

Effects of Sonic Disruption and Ashing of Cells

A test involving 128 insects was conducted in which *B. circulans* cells were disrupted by sonic vibration before incorporation into the diets. Cells grown in the manner previously described were suspended in distilled water and disrupted by 30 minutes' exposure in a 10-kc Raytheon magnetostrictive oscillator.[6]

Sonic disruption did not affect the accelerating activity of the cells. Larvae developed to the third instar in an average of 7.2 days on the control diet, in 5.4 days on diet plus whole nonviable *B. circulans* cells, and in 5.7 days on diet plus disrupted cells. No significant loss in activity was caused by the sonic treatment, indicating that no significant denaturation of the active factors occurred.

Further tests were conducted to determine whether accelerating activity was due to inorganic constituents of the bacterial cells. Dried *B. cereus* cells were ashed in a muffle furnace and the ash incorporated into the diet. Addition of the ash from 6.6 mg and 13.2 mg of dried cells to each milliliter of diet failed to exhibit the accelerating activity, indicating that stimulation was not due to a simple salt effect.

Addition of Dialyzate of B. cereus

To determine whether the accelerating activity was due to some dialyzable component of the bacterial cell, various levels of dialyzate were added to

[6]Raytheon Manufacturing Co., Waltham, Mass.

tubes of diet. The dialyzate was prepared as follows: 2 g of freeze-dried *B. cereus* cells was suspended in 20 ml of distilled water, sonerated for 20 minutes, and dialyzed against 6 liters of distilled water at 6° C for 24 hours. The dialyzate was concentrated to 5 ml on a rotary evaporatory at 50° C, and added to diet tubes containing the control diet. Four concentrations of dialyzate were tested: 3.3, 6.6, 13.2, and 26.4 mg equivalents of dried cells per milliliter of diet.

All the levels of dialyzate caused a significant acceleration in development but the accelerating potency was extremely low (Table II). The greatest acceleration, 0.9 days, occurred when dialyzate equivalent to that obtainable from 26.4 mg of dry cells was added to each milliliter of diet. The activity of the dialyzate indicates that at least part of the acceleration is caused by a water-soluble substance of a relatively low molecular weight. The low accelerating potency further suggests that the effect may be due to a complex of factors, only part of which is dialyzable.

TABLE II

Development of *H. antiqua* larvae on a chemical diet supplemented with various concentrations of *B. cereus* dialyzate

Dialyzate supplements (mg equivalents of *B. cereus* dialyzate per ml of diet)	Number tested	Days from hatching to reach			
		Third instar		Pupa	
		Av.	Range	Av.	Range
None (control)	26	7.3	6–9	14.6	12–20
3.3	15	6.7*	6–8	13.3	12–15
6.6	17	6.6*	6–8	13.6	12–17
13.2	14	6.6*	6–8	13.9	11–15
26.4	16	6.4**	6–8	12.9	12–15

**,*Significantly different from control; necessary difference, $P = 0.01 = 0.81$ days, $P = 0.05 = 0.60$ days.

Effects of Addition of Casein

It was realized during the dosage-effect studies with dried *B. cereus* cells that appreciable amounts of protein were being incorporated into the diet when 8 mg of cells was added per milliliter of diet. Sang (5) showed that

TABLE III

Development of *H. antiqua* larvae on a chemical diet supplemented with various levels of casein

Casein supplements (mg per ml of diet)	Number tested	Days from hatching to reach			
		Third instar		Pupa	
		Av.	Range	Av.	Range
None (control)†	43	6.9	5–8	13.8	13–17
1	37	6.8	6–9	14.0	12–18
2	49	6.7	5–8	13.4	11–16
4	36	6.3*	5–8	13.6	12–17
8	33	5.9*	5–9	12.7	12–15

*Significantly different from control; necessary difference, $P = 0.01 = 0.4$ days.
†Contained 24.21 mg of amino acids per milliliter of diet.

TABLE IV

Lengths of *H. antiqua* larvae at certain physiological stages when grown on a chemical diet under axenic and monoxenic conditions

Diet	One day before third instar					Three days before pupation				
	Number measured	Days from hatching		Length in mm		Number measured	Days from hatching		Length in mm	
		Av.	Range	Av.	Range		Av.	Range	Av.	Range
Axenic	24	6.8	5–8	4.5	3.7–5.8	30	13.3	11–18	8.5	7.3–9.5
Monoxenic with living *B. circulans*	11	3.3	3–4	4.4	3.7–4.8	29	6.8	6–9	8.4	6.2–10.1

the protein level in the diet of *Drosophila melanogaster* (Meig.) was very critical. He found that the larval period was reduced by 1.5 days when the protein level rose from 1.5 to 2% and by 0.2 days when the level rose from 2.5 to 3%. The addition of 8 mg of dried *B. cereus* cells to each milliliter of diet in the present experiments would raise the protein level of the diet by at least 0.5%. If the 2.4% amino acid content of the diet is too low for optimal development the addition of 0.5% protein might accelerate the development rate of *H. antiqua* larvae significantly.

A test was conducted to determine the effects of addition of casein on larval development. The diet was supplemented with various levels of Genatosan,[7] low vitamin casein used by Sang (5). Table III shows that additions of 4 mg and 8 mg per milliliter of diet accelerated the development from hatching to the third instar. Since 8 mg per milliliter of dried *B. cereus* cells accelerated development by 2 days (Table II) and the same amount of casein accelerated development by 1 day, the casein exhibits about one-half the accelerating potency of the bacterial cells. The fact that casein supplements accelerated the development suggests that the acceleration effect may be due, in part, to a more optimal nutritional balance.

Measurements of Accelerated and Nonaccelerated Larvae

Daily measurements were made of the length of larvae on the control diet and on a diet inoculated with *B. circulans*. The measurements were made with an optical micrometer having a maximum error of 0.4 mm.

Table IV shows that both the growth rate and the rate of metamorphosis were accelerated by the presence of *B. circulans*; when the lengths of larvae from the two diets were compared at similar physiological stages, however, they did not differ significantly. It appears that a critical minimum size is reached before *H. antiqua* metamorphoses into a more adult form. At an average of 6.8 days, larvae on the control diet only averaged 4.5 mm in length compared with 8.4 mm for the monoxenically reared larvae. The larger larvae were more nearly mature, however, and pupated in 3 days in comparison with 6 days needed by the axenically reared larvae.

Discussion

In previous studies (3) on the nutritional requirements of larvae of *H. antiqua*, chemical diets were developed on which the larvae could be reared successfully under aseptic conditions. An accelerated development rate occurred when the diets were contaminated by various bacteria. It was frequently noted that contaminated areas of the diet were fed on in preference to other parts, suggesting that the microorganisms contained or produced some substance(s) that was accelerating the development rate of the larvae.

The present studies have shown that a variety of bacterial isolates can produce this effect and that the active factor(s) is fairly heat-stable, will withstand sonic treatment, is partially dialyzable, and is an intrinsic part

[7]Genatosan Ltd., Loughborough, Leicestershire, England.

of the bacterial cell. That additions of purified casein to the diet also produced a significant stimulation in larval development suggests that at least part of the accelerating factor(s) is proteinaceous. Autoclaving the bacterial cells might denature some of the cellular proteins, thus explaining the lowered accelerating potency in nonviable cells. No protein molecules would be present in the dialyzate of the cells but certain polypeptides and amino acids could penetrate the membrane and account for the observed activity of the dialyzates.

It is possible that the proteins and polypeptides supplied by the bacteria may be more readily and efficiently utilized by the larvae than those provided in the basic chemical diet. The accelerating potency of the nonviable cells of *B. circulans* and *B. cereus* and of the casein might be due to qualitative and quantitative differences in the relative concentrations of amino acids in these cellular proteins. Small changes in relative amino acid concentrations have been shown to have gross effects on the rate of development of *H. antiqua* larvae (3).

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OSMOREGULATORY ADAPTATION OF STEELHEAD TROUT (SALMO GAIRDNERI RICHARDSON) TO SEA WATER[1]

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Abstract

Steelhead trout transferred from fresh water into sea water exhibited two distinct phases in osmoregulatory adaptation. The first, or adjustive phase, was distinguished by marked departures from fresh-water levels and distributions of water and electrolytes. Increases in muscle chloride space indicated transfer of fluids from cells into the extracellular compartment. Plasma levels of water, chloride, sodium, and potassium rose, while that of calcium remained relatively constant. The ratio of plasma sodium to chloride fell sharply suggesting a condition of acidosis. Cellular calcium concentrations rose markedly, while smaller increases were seen in chloride and potassium levels. Changes in calcium appeared to be a function of both cellular dehydration and electrolyte uptake. Changes in cellular potassium concentrations were related primarily to dehydration since tissue levels of this ion fell slightly in sea water. The roles of active electrolyte excretory processes, and the passive withdrawal of ions from the circulating fluids by complex formation, have been considered as osmoregulatory mechanisms leading to the establishment of the second, or regulative, phase of adaptation to sea water.

Introduction

Because steelhead trout are anadromous in habit the juvenile migrants must adapt within relatively short periods to major increases in the salt content of their environment. It has been abundantly demonstrated that salmonids, as well as other migratory species, have the ability to accomplish this conversion rapidly (3, 15, 23). Nevertheless adaptation to sea water is invariably accompanied by an initial adjustive period (35), during which the fish must tolerate increased plasma and tissue electrolyte levels, and losses of body water.

Studies carried out thus far on the adaptation of migratory fish to sea water have dealt primarily with over-all changes of osmotic pressure, and of water and inorganic electrolyte concentrations of plasma and tissue. Little consideration has been given to possible variations in body fluid distribution and attendant translocations of electrolytes. It has been the purpose of the present investigation to clarify the effect of transfer from fresh water into sea water on the distribution of body water, and to determine the roles of the extracellular and intracellular compartments in accounting for observed changes in tissue electrolyte concentrations.

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Materials and Methods

Experimental Stocks

Juvenile steelhead trout used in these studies were obtained from the Smith's Falls Hatchery of the British Columbia Game Commission at Cultus Lake, British Columbia. The fish were maintained at the University in running water and fed a dry commercial diet (Clark's Fish Food)[3] similar to that used in their original hatchery. The water supply consisted of dechlorinated city water, and varied from 10 to 16° C over the period of maintenance. The fish were subjected to normal spring and summer day lengths. Following the usual "delayed mortality" commonly found after transportation of fish, few deaths occurred in the experimental stocks during the investigation.

Acclimation of Trout to Sea Water

The water–electrolyte metabolism of fish has been shown to respond sharply to starvation (22), handling (16, 29), and forced activity (1, 2). Accordingly the method of acclimating the trout to sea water was designed to avoid these factors as far as possible.

Wooden boxes of approximately 50 liters capacity were weighted and floated in constant temperature baths (10.0 ± 0.2° C). Insulated dark covers reduced visual stimulation, while the surrounding water bath dampened auditory stimuli and mechanical shocks. The tanks were aerated throughout acclimation, and the fish at 12-hour intervals. About 20 liters of fresh water was added to each box and time allowed for temperature equilibration. Six to ten trout were then transferred in water from the hatchery troughs, and fresh water added to a depth of 20 cm (approximately 40 liters). The boxes were covered, and an interval of 36 to 40 hours allowed for recovery of the fish from any shock of handling, and for acclimation to the experimental temperature. The studies of Black (2) indicate that this was an ample recovery period considering the small degree of activity induced in the animals during transfer. Following this interval water was withdrawn by means of a fixed siphon to a depth of 8 cm, and the tanks refilled with sea water from an overhead reservoir. Sea-water concentrations were adjusted such that a final salinity of 22 to 24‰ was achieved in the acclimation tanks. Little disturbance was noted in the fish during this procedure, and it was believed that experimentally induced variations in water–electrolyte patterns had been avoided.

Sampling

Prior to sampling, fish were anesthetized in a solution of tricainemethanesulphonate.[4] Individual fish were wrapped in damp towelling, and loosely held with rubber bands, ventral side uppermost, upon a frame. The heart was exposed ventrally to avoid contamination of samples with pericardial fluids and blood drawn from the bulbus arteriosus into heparinized syringes.

[3]This food was generously supplied by Mr. J. R. Clarke, Salt Lake City, Utah.
[4]MS 222, Sandoz Co., Basle, Switzerland.

Mild massage from the caudal region forward aided in the expression of blood. Samples were immediately chilled in an ice bath, and thereafter centrifuged. Hemolyzed samples were discarded.

Tissue was excised from the dorsal fin area of the epaxial muscle band, rapidly dissected free of cartilage, skin, bone, and fat, and immediately stored in air-tight, moist chambers. When kept in these containers, tissue samples could be held for several hours with no appreciable water loss. Plasma samples were capped with Parafilm to reduce evaporation, and both they and muscle samples refrigerated until used.

Analytical Techniques

Plasma chloride concentrations were determined on 0.1-ml samples by the method of Schales and Schales (37). Tissue chloride was determined by the modified "open Carius" procedure proposed by J. F. Manery (1955, personal communication). In this technique dried tissue samples are soaked overnight in silver nitrate, digested in nitric acid over a boiling water bath, and the residual silver nitrate determined by thiocyanate titration. Closure of the digestion tubes with glass bulbs having downward-drawn tips minimized loss of chloride through the formation of volatile chloride-containing compounds during the process of digestion (38).

Plasma calcium concentrations were obtained by the method of Lehmann (26) using a Klett-Summerson photoelectric colorimeter and 500 μ second order interference filter. Determinations were carried out on 0.1-ml samples. Tissue calcium values were estimated by the procedure of Comar, Hansard, Hood, Plumlee, and Barrentine (6).

Plasma and tissue concentrations of sodium and potassium were determined with a Janke flame spectrophotometer, using lithium as an internal standard. Tissue samples were digested in nitric acid, with the addition of small aliquots of hydrogen peroxide, redissolved in dilute sulphuric acid, diluted to about 1:200 with 10% aqueous lithium nitrate, and read against bracketing sodium and potassium chloride standards. Plasma samples were diluted 1:200 in the same solution and estimated without prior digestion.

Water concentrations were obtained by drying plasma and tissue samples to constant weight at 103° C.

Estimation of Extracellular Phase

The ready disturbance of electrolyte metabolism in fish (1, 2, 16, 22, 29) and the lack of constancy in values determined during serial sampling trials indicated that estimates of extracellular volume based upon substances requiring prior injection into the animals would probably not be valid. Estimates of this phase have been made largely by the chloride space technique, and in the case of certain larger fish by Conway's "chloride–potassium" approach as well (7), with the realization that the phase described in this way might overlap the cellular compartment to some extent. That this overlap was slight was indicated by the fact that intracellular volumes calculated by both techniques varied by only 3 to 6% (see Results).

Using Manery's definition of the chloride space (27) as "the volume required to distribute all the tissue chloride at the concentration of a plasma ultrafiltrate", the extracellular phase may be estimated as follows:

$$(1) \qquad ECS = \frac{(t\mathrm{Cl})\ (r\mathrm{Cl})\ (p\mathrm{H_2O})}{(p\mathrm{Cl})}$$

where t = tissue concentration, p = plasma concentration, and $r\mathrm{Cl} = 0.977$.

The approach favored by Conway (7) depends upon the validity of the assumption that:

$$(2) \qquad (ecs\mathrm{K})\ (ecs\mathrm{Cl}) = (ics\mathrm{K})\ (ics\mathrm{Cl})$$

where ecs = extracellular phase concentration and ics = intracellular phase concentration, and is summarized by the equation:

$$(3) \qquad ECS = \frac{(t\mathrm{K})\ (t\mathrm{Cl}) - (t\mathrm{H_2O})^2\ (ecs\mathrm{K})\ (ecs\mathrm{Cl})}{(ecs\mathrm{K})\ (ecs\mathrm{Cl}) + (t\mathrm{K})\ (t\mathrm{Cl}) - 2(t\mathrm{H_2O})\ (ecs\mathrm{K})\ (ecs\mathrm{Cl})}.$$

Intracellular volumes were determined as the difference between total tissue water and extracellular phase. Estimates of extracellular phase electrolyte concentrations were made using formulae provided by Manery (27), and the Donnan factors listed by this author for chloride, sodium, potassium, and calcium. Cellular ion levels were calculated by subtracting the amount of electrolyte present in the extracellular compartment from the total tissue concentration. Cellular concentrations have been expressed per liter cell water and/or per kilogram tissue solids.

Results

Simultaneous analyses of plasma and muscle chloride and water concentrations were carried out on trout in fresh water, and after periods of 4, 10, 15, 22, 28, 36, 86, 126, and for plasma chloride only, 168 hours in sea water. Two groups drawn from the same population were used. The first, sampled in June and July, 1957, consisted of fish varying in weight from 25 to 70 g. The second, sampled during February and March, 1958, included fish in a weight range of 70 to 250 g. The fish of the latter group were in some cases sufficiently large that plasma and tissue sodium and potassium values as well as chloride and water concentrations could be determined. In the latter instance fish were sampled in fresh water, and after 36 hours in sea water.

Tissue and plasma water and chloride values were plotted against weight, and regression equations calculated for groups in sea water and in fresh water. In the following figures the values for fresh water and 36-hour sea-water groups are based only on the fish tested in June and July, 1957. Since weight distributions varied to some extent in the individual groups, values for 55-g fish (mean population weight) have been interpolated from the equations for plotting unless otherwise stated. In the figures, numbers in parentheses indicate the sample size, while the vertical lines represent one standard error of regression. The effect of weight as a factor influencing the adaptability of steelhead trout to sea water will be considered in a later publication.

Plasma Chloride

Sixty-two steelhead were sampled to establish variations in fresh-water plasma chloride levels. Marked decreases in plasma chloride concentration with increase in weight were evident in fish sampled from June to July, 1957 (Table I). In fish between 25 and 40 g in weight plasma chloride concentrations fell from about 160 mmole/l. to approximately 140 mmole/l. A slight additional decrease was seen with weight increases from 50 to 250 g. The high values for fish weighing less than 40 g could not have been due to evaporation of water from samples since the plasma water levels of the fish in question were as high or higher than those of animals exhibiting lower chloremias (Table I). Kubo (25) has noted similar variations in plasma chloride levels in the *Sakuramasu* (*Oncorhynchus masou*). Early smolt stages were characterized by high plasma chloride concentrations relative to late stage parr. Late smolts exhibited falling chloride levels. The steelhead trout appears to resemble the *Sakuramasu* in this respect. Those with unusually high values probably correspond to Kubo's early smolt stages. No early or late stage parr were sampled in this programme.

Figure 1 summarizes changes in plasma chloride concentrations of 55-g trout following their transfer into sea water. An immediate rise in chloride concentration occurred and a peak was reached after 36 hours in sea water. From 36 to 168 hours after transfer chloride levels fell smoothly, and at the last period of sampling were approximately at the fresh-water level.

Tissue Chloride

Tissue chloride concentrations were determined on 58 fresh-water fish. Smaller animals contained considerably more chloride per kilogram fresh tissue than did larger trout, but unlike the situation in plasma levels no abrupt weight-related variations were seen. A steady decrease from about 28 to 10 mmole/kg occurred between 30 and 110 g. Thereafter tissue levels were relatively steady at between 8 and 13 mmole/kg.

Transfer into sea water was accompanied by an immediate increase in tissue chloride content (Fig. 2). A peak level was reached 36 hours after transfer and coincided with the peak for plasma concentration. In both instances two phases of uptake were apparent. Rapid changes took place during the initial 4 hours for tissue and for the initial 10 hours for plasma, when the net plasma rate of change was 2.24 mmole/l./hour, and that for tissue was 2.49 mmole/kg/hour. Between these periods and 36 hours after transfer the rates of uptake were much less: 0.18 mmole/kg/hour for tissue, 0.45 mmole/l./hour for plasma. Stable tissue concentrations were reached after 86 hours in sea water, and were about 10% above pre-transfer concentrations.

Plasma Water

Plasma water levels in fresh water were determined for 58 trout. Again a size-related variation was apparent (Table I). Unlike plasma chloride, plasma water concentrations did not fall abruptly between 30 and 40 g. A steady

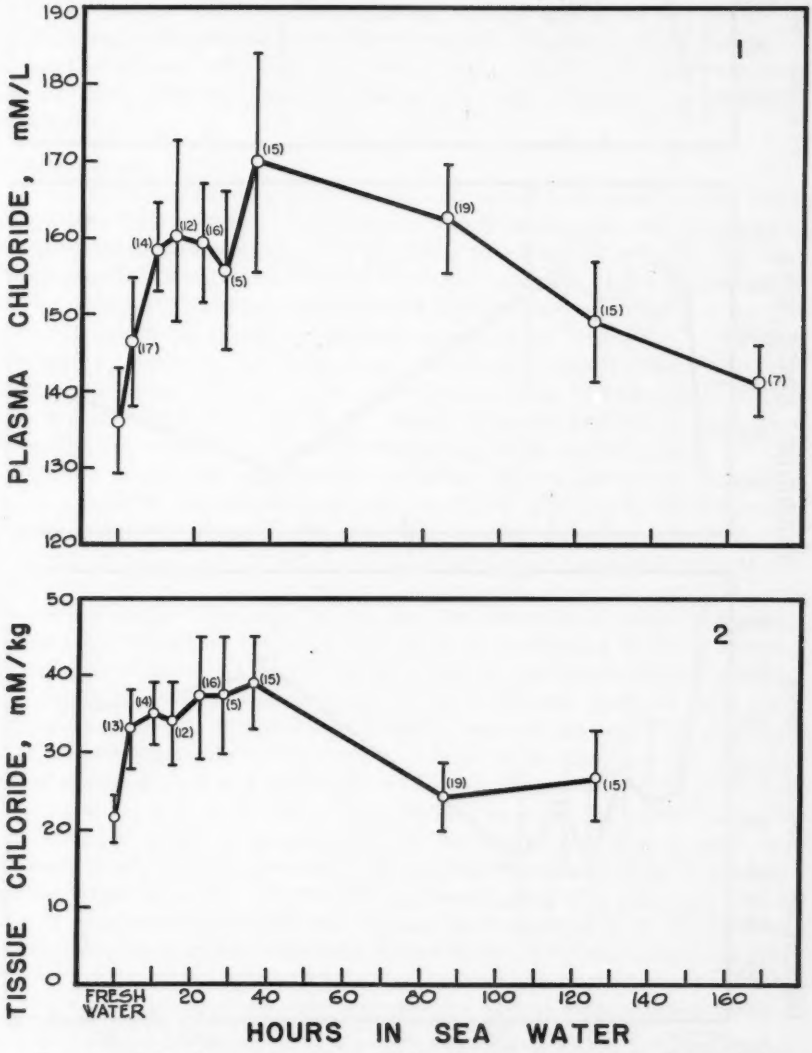
TABLE I

Variation of plasma and tissue chloride and calcium, plasma water, chloride space, and cellular space (tissue water – chloride space) with weight in fresh-water steelhead

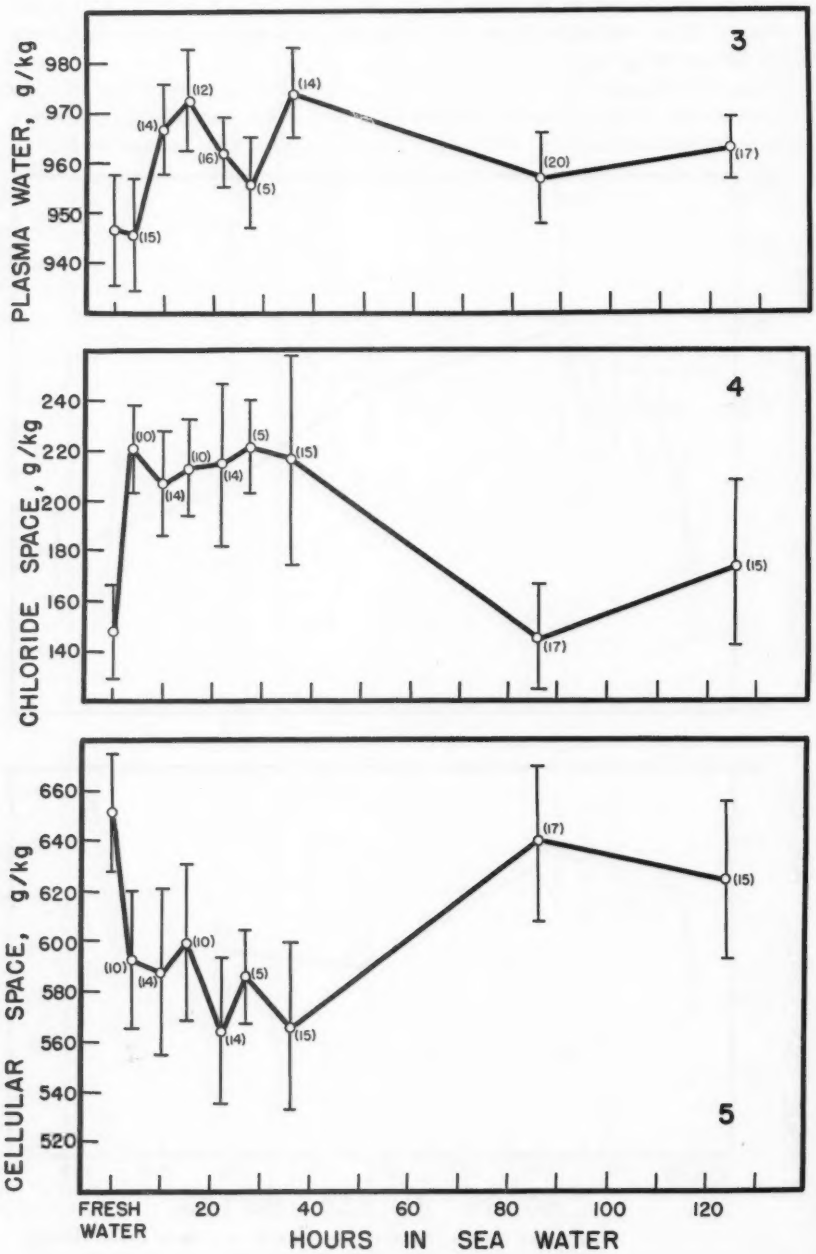
	June–July, 1957			February–March, 1958		
Constituent	Sample size	Weight range, g	Regression equation	Sample size	Weight range, g	Regression equation
Plasma chloride	39	25–70	$Y = 152.8 - 0.30X$	23	76–250	$Y = 141.0 - 0.04X$
Plasma water	39	25–70	$Y = 958.4 - 0.22X$	19	76–250	$Y = 934.3 - 0.016X$
Plasma calcium	24	47–118	$Y = 2.71 - 0.004X$			
Tissue chloride	35	30–70	$Y = 31.9 - 0.18X$	23	80–250	$Y = 14.9 - 0.02X$
Tissue calcium	23	47–118	$Y = 3.24 - 0.015X$			
Chloride space	33	32–70	$Y = 217.1 - 1.28X$	19	80–250	$Y = 103.4 - 0.156X$
Cellular space	33	32–70	$Y = 628.3 + 0.43X$	19	80–250	$Y = 619.2 - 0.023X$

downward trend occurred between 30 and about 100 g. In fish heavier than 100 g, plasma water concentrations were relatively size-stable, and varied from about 920 to 945 g/kg.

Figure 3 indicates variations in plasma water content following transfer into sea water. Unlike plasma chloride concentrations, those of plasma water showed no immediate rise. Following an abrupt increase in environmental



FIGS. 1 and 2. Changes in plasma chloride (Fig. 1) and in muscle tissue chloride (Fig. 2) concentrations of trout following transfer from fresh water into sea water (values interpolated for 55-g fish).


3
PLASMA WATER, g/kg
980
970
960
950
940
(14)
(12)
(16)
(5)
(14)
(15)
(20)
(17)
4
CHLORIDE SPACE, g/kg
240
220
200
180
160
140
(10)
(14)
(10)
(14)
(5)
(15)
(17)
(15)
5
CELLULAR SPACE, g/kg
660
640
620
600
580
560
540
520
(10)
(14)
(10)
(14)
(5)
(15)
(17)
(15)
FRESH WATER
20
40
60
80
100
120
HOURS IN SEA WATER

tonicity, changes in plasma water content must be the net of two opposing processes: depletion due to osmotic transfer from circulating fluids to environment, increases resulting from the transfer of fluid from cells to the extracellular compartment. The level at 4 hours suggested that these two processes were in equilibrium at that time; subsequent increases in water concentrations may indicate that transfer was more rapid than loss.

Unlike plasma chloride concentrations, plasma water did not return to fresh water levels over the experimental period. The mean water content of samples at 86 and 126 hours was 959.6 g/kg, a value 1.4% above the pre-transfer level. This net increase in plasma water was, however, not statistically significant.

Chloride Space

Table I indicates the variation of chloride space with weight in 52 trout sampled in fresh water. The volume of the chloride space fell from about 165 g/kg in animals weighing less than 40 g to about 130 g/kg in 70-g fish. Volumes of between 50 and 90 g/kg were characteristic of trout heavier than 100 g. A similar decrease, though much less marked, was obtained by the recalculation, by a simplified method suggested by Conway (7), of data provided by Fontaine, Callamand, and Vibert (14), and Fontaine (12), for plasma and tissue chloride values of Atlantic salmon (*Salmo salar*). Early parr had chloride spaces of 83 g/kg. Mean values for late parr ranged from 79 to 80 g/kg, while that of actively migrating smolts was 64 g/kg.

Figure 4 summarizes variations in the volume of the chloride space accompanying transfer of steelhead trout into sea water. Changes in chloride space occurred in three phases: an initial rapid increase in volume immediately following entry into sea water, a second stage during which volume was relatively steady, and finally a return of the chloride space to approximately fresh-water levels. The mean of the last two observations was 158.8 g/kg, a level about 8% above the value for fresh-water animals of the same weight. An estimate of the net rates of increase in chloride space volume over the first two phases of increase was made by regressing chloride space on time over the period from 4 to 36 hours after transfer when volume was relatively constant. From the value interpolated at 4 hours from this equation, the net rate of change from 0 to 4 hours was +17.9 g/kg/hour. That between 4 and 36 hours was +0.12 g/kg/hour. The rapid phase of change probably results from chloride uptake uncompensated by chloride excretion, since some lag has been found in the activation of the excretory mechanisms after transfer into sea water (8, 9, 18). The second and slower phase may then represent a type of quasi-equilibrium between uptake and excretion with the balance slightly in favor of uptake. Between 36 and 86 hours the decrease in chloride

FIG. 3. Changes in plasma water concentrations of trout following transfer from fresh water into sea water (values interpolated for 55-g fish).

FIG. 4. Changes in chloride space of trout muscle following transfer from fresh water into sea water (values interpolated for 55-g fish).

FIG. 5. Changes in cellular space (tissue water – chloride space) of trout muscle following transfer from fresh water into sea water (values interpolated for 55-g fish).

space suggests that the balance had shifted to excretion, while during the regulative phase a dynamic equilibrium between uptake and excretion would be expected.

Chloride–Potassium Space

Trout sampled in February and March varied in weight from 70 to 250 g and some were sufficiently large that both plasma and tissue chloride and potassium levels could be established on individual fish. Comparisons were made of the absolute values for chloride and chloride–potassium spaces, and of changes in volume resulting from transfer of the trout into sea water.

In this group of fish, little weight-related variation in electrolyte concentrations was encountered in either plasma or tissue. On the basis of this observation, therefore, sample means have been reported rather than values interpolated from regression equations for fish of standard size.

The mean chloride space of fresh-water trout was 87.8 g/kg, while that of the chloride–potassium space was 63.1 g/kg. The discrepancy between these two values may be attributed to the inclusion of chloride in the intracellular phase by the second technique. The variation in cellular water volumes predicted on the basis of the two approaches was 3.6%. The corresponding volumes for trout after 36 hours in sea water were 169.3 g/kg (chloride space) and 138.1 g/kg (chloride–potassium space). The difference in predicted intracellular volumes was 5.5%.

These data indicate that some variation in estimates of extracellular volume arises from the presence of chloride within muscle fibers. This might be expected, but nevertheless the pattern of results indicated for the effect of transfer into sea water on the volume of this phase was essentially the same. The increase in the chloride space was 81.5 g/kg, while that of the chloride–potassium space was 75.0 g/kg. It would seem probable therefore that the chloride space adequately describes *changes* in the volume of the extracellular phase following entry of the fish into sea water.

Comparison of extracellular volumes in fish in sea water with those of fresh-water trout depends upon the validity of the assumption that entry into sea water is not accompanied by an increase in the total amount of chloride contained in the muscle fibers. Usage of chloride – potassium space values allowed the estimation of changes in intracellular chloride concentrations, and of the amount of cell-associated chloride. The concentration of cellular chloride derived for fresh-water fish was 5.5 mmole/l., a value in good agreement with those cited by Conway (7) for amphibian and mammalian muscle. The level for fish after 36 hours in sea water was 7 mmole/l. The total amounts of cellular chloride, however, did not vary significantly due to the decrease in intracellular volume (3.9 mmole/kg in fresh water, 4.1 mmole/kg in sea water).

Intracellular Space (Tissue Water – Chloride Space)

Table I includes equations describing changes in volume of the intracellular space with weight in fresh-water steelhead. Despite considerable scatter a trend toward higher cellular volumes in heavier animals was apparent.

Figure 5 summarizes changes in the volume of the cellular phase following transfer into sea water. As with the extracellular compartment, changes in the volume of this phase were divisible into initial fast and slow components, and subsequent return to about the fresh-water level. The mean of points taken at 86 and 126 hours was 2.1% below the fresh-water value. By the procedure outlined for changes in chloride space, rates of change in cellular phase volume were calculated to be −16.9 g/kg/hour and −0.83 g/kg/hour over the initial fast and slow phases of change respectively. Loss of water from the cellular compartment may, therefore, be attributed first to its transfer into the extracellular phase, and secondly to over-all losses to the environment. The close correspondence of values for increase in extracellular and decrease in intracellular phases suggests that net water loss may have been relatively slight in comparison to translocation from one compartment to the other. During the second phase the data suggest that water loss from the animals to their environment exceeded transfer from cellular to extracellular compartments. The rate of over-all loss, estimated as the difference between rates of increase and decrease in the phase volumes, was −0.71 g/kg/hour.

Seasonal Variations in Adjustive Responses to Sea Water

Seasonal variations in the effects of environmental variables on fish have frequently been demonstrated (e.g., 4,21). If valid comparisons are to be made between data derived from the June–July and February–March samples this possibility must be considered. Table II compares changes in plasma water, plasma and tissue chloride, chloride and chloride–potassium spaces, and cellular phases based upon these spaces in the two groups. Good agreement is seen between values interpolated for the larger fish sampled in June and July (70 g) and the means for the February–March group, except in the instance of plasma water where a reversal in direction of change took place. Notwithstanding this discrepancy, the bulk of the data indicate that despite

TABLE II

Adjustive responses to sea water (36 hours) in June–July and February–March groups of steelhead trout

Constituent	Change in concentration or volume after 36 hours in sea water	
	June–July group*	February–March group†
Plasma chloride	+36.4 mmole/l.	+27.4 mmole/l.
Tissue chloride	+15.7 mmole/kg	+18.2 mmole/kg
Plasma water	+27.8 g/kg	−19.2 g/kg
Chloride space	+69.9 g/kg	+81.5 g/kg
Chloride/potassium space	—	+75.0 g/kg
Tissue water – chloride space	−93.9 g/kg	−94.1 g/kg
Tissue water – chloride/potassium space	—	−87.7 g/kg

*Values interpolated for 70-g trout.
†Mean values for samples.

growth and possible changes in electrolyte metabolism transfer into sea water produced essentially the same type and extent of response. Comparisons between the data derived from the two groups are therefore considered to be reasonable.

Potassium and Sodium

Of the 60 fish sampled during February and March, 1958, values were obtained as follows: fresh water: plasma chloride −23, plasma water −24, plasma sodium and potassium −24, tissue chloride −21, tissue potassium −12; after 36 hours in sea water: plasma chloride −23, plasma water −25, plasma sodium and potassium −22, tissue chloride −35, tissue potassium −18. Cellular potassium values were calculated for fish on which complete sets of data were available. Such concentrations are reported for 11 fish in fresh water, and 13 fish in sea water.

(*a*) *Sodium*

The mean plasma sodium concentration of trout in fresh water was 162.5 mmole/l.; that of fish in sea water for 36 hours was slightly higher, 170.0 mmole/l. The mean sodium–chloride ratio for fresh-water animals was 1.202, a value in good agreement with ratios calculated from data provided by Field, Elvehjem, and Juday (11), and Forster and Berglund (16), for fresh-water and marine fish. Despite the rise in plasma sodium levels the mean sodium–chloride ratio fell to 1.046 in sea water. The data suggest a departure from electroneutrality in the extracellular fluids. The investigations of Fontaine and Boucher-Firly (13), however, indicate that adaptation to sea water may be accompanied by reduction of plasma bicarbonate concentrations which would tend to alleviate the establishment of an anionic excess in the plasma. Decreased plasma bicarbonate concentrations may, however, result in a condition of acidosis in the migrants. This may have been a true acidosis resulting solely from a decrease in the bicarbonate concentration of the blood, or it may have been a compensated acidosis in which the fall in bicarbonate ion was paralleled by a fall in carbonic ion as well. The variation in sodium–chloride ratio may be accounted for in a number of ways, all of which turn upon various mechanisms whereby fish may reduce the amount of circulating sodium in their extracellular compartments. Active excretion of sodium through the gills has been described (24, 30). Active transport of chloride may be initiated more slowly, or proceed at a lesser rate than that of sodium, and should be considered as a possible explanation. Sodium may also be withdrawn from the circulating fluids by complex formation with either soft tissues or with bone.

(*b*) *Potassium*

The mean plasma potassium concentration of steelhead trout in fresh water was 6.04 mmole/l. Corresponding concentrations for tissue and cells were 125.2 mmole/kg and 182.0 mmole/l. cell water respectively in the same medium. The latter values are somewhat greater than those reported by Nishie and Harris (33) for *Electrophorus*, but the tissue levels correspond well with those given by McBride and MacLeod (28) for various species of Pacific salmon.

In movement from fresh water into sea water the fish meet an environment in which the increase in sodium concentration is high relative to the increase in potassium. Elkington and Danowski (10) note sodium–potassium ratios of 100:34–45 in fresh water, and 100:2 in sea water. Similarly Sverdrup, Johnson, and Fleming (39) give ratios of 100:40 and 100:3 for these media. Since both ions enter fish under appropriate concentration gradients, a relatively greater enrichment of sodium than of potassium might be expected to occur in the body fluids. Under such circumstances it has been noted that cellular sodium levels increase while cellular potassium falls (17). The decrease in mean tissue potassium concentration from 125.2 to 116.2 mmole/kg was in accordance with the observations cited above. Cellular potassium levels, on the other hand, rose from a mean of 182.0 mmole/l. cell water to a mean of 192.0 mmole/l. cell water. This apparently anomalous situation was clarified by recalculation of cellular potassium concentrations on the basis of fresh-water intracellular phase volumes. The value obtained in this way, 168.1 mmole/l. cell water, was in line with the observed decrease in tissue concentrations. The noted rise in cellular concentrations of this cation may be attributed, therefore, to the concentration of potassium remaining in the cells by cellular dehydration.

Concomitant with transfer into sea water, plasma potassium levels rose from a mean of 6.04 mmole/l. to 7.61 mmole/l. This enrichment of the fluid phases may be related to recruitment of potassium either from the environment or from the cellular pool.

Calcium

(*a*) *Plasma*

A sample of 24 trout was used to establish fresh-water plasma calcium concentrations. The values obtained exhibited some weight-dependence, but much less than that observed for plasma chloride, ranging from 2.6 mmole/l. for 40-g fish to 2.4 mmole/l. for 90-g trout. The standard error of regression, 0.32 mmole/l., was much smaller than that for either plasma chloride (8.4 mmole/l.) or sodium (5.9 mmole/l.), suggesting the influence of a relatively efficient plasma calcium regulatory mechanism.

The size range of animals tested, 47 to 116 g, did not extend low enough to indicate any variation which might be attributed to parr–smolt transformation. Data reported by Fontaine, Callamand, and Vibert (14), however, suggest the occurrence of such variations in this stage of the life history of the Atlantic salmon.

Plasma and extracellular fluid calcium concentrations following transfer into sea water were notable primarily for their constancy (Fig. 6). Plasma levels rose slightly between 52 and 168 hours, while extracellular concentrations decreased somewhat between 15 and 26 hours after transfer. In neither case, however, were the changes statistically significant.

The constancy of fluid phase calcium concentrations in sea water differed markedly from the situation encountered with plasma chloride and sodium under the same conditions. Since transfer into sea water of the salinity used

in these investigations involved a 10 to 15 times increase in environmental calcium concentrations, two possible explanations may be advanced: either calcium was prevented from entering the fish, or an extremely efficient extracellular phase regulatory system operated in the trout. Data presented in the following paragraphs indicate that calcium did, in fact, enter the fish. The second alternative, regulation, will be discussed in a later section.

(*b*) *Tissue*

Tissue calcium levels were determined on 24 fresh-water trout, and as with most other electrolytes and water, a decrease in concentration with increase in body weight was apparent. Since there was also some decrease in the size of the extracellular phase with increase in weight the lowering of tissue levels in heavier animals may have been the reflection of a lessened contribution of extracellular phase calcium to the total tissue levels.

An estimate of cellular phase calcium made by subtracting the amount contained in the extracellular phase from the tissue concentration did not indicate any levelling of values. When expressed either per liter cell water, or per kilogram tissue solids, the variation with weight became more apparent.

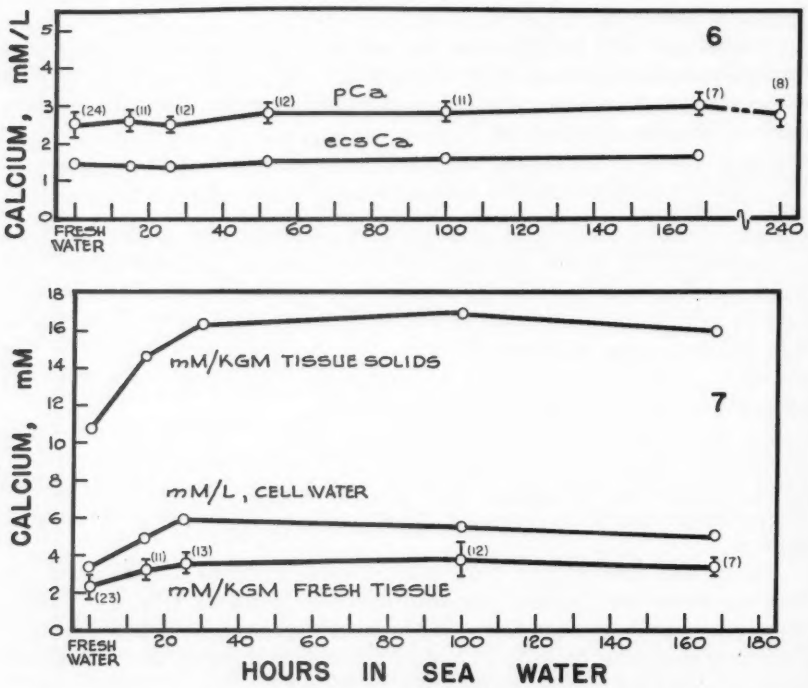


FIG. 6. Changes in plasma and extracellular fluid calcium concentrations in trout transferred from fresh water into sea water (values interpolated for 55-g fish).

FIG. 7. Changes in tissue and cellular calcium concentrations of trout muscle following transfer from fresh water into sea water (values interpolated for 55-g fish).

Following transfer into sea water a sharp rise in tissue calcium concentrations was noted (Fig. 7). A similar rise in cellular calcium was also apparent (Fig. 7). To some extent, and particularly when cellular levels are reported per liter cell water, the increase in concentration may be accounted for by the concentration of previously present calcium through cellular dehydration. Recalculation of cellular values on the basis of fresh-water intracellular volumes, however, indicated that this effect would not account for more than 15% of the observed change in concentration. A real, as opposed to apparent, increase in concentration must, therefore, have occurred.

Discussion

Plasma electrolyte concentrations obtained in this study for fresh-water trout agree well with those reported by earlier authors for salmonids (Table III). Tissue chloride values reported in the literature are, in general, lower than those obtained in this study (e.g., 12, 19). The discrepancies may

TABLE III

Representative plasma electrolyte levels of salmonid species

Electrolyte	Species	Plasma concentration, mmole/l.	Reference	Remarks
Chloride	*Oncorhynchus masou*	110.0	30	Smolting parr
		119.8		Early smolt
		110.0		Late smolt
		162.2		Sea water*
	Salmo trutta	119.4	11	Fresh water
	Salmo salar	123.1	14	Parr
		122.6		Smolt
		135.5		Fresh run adult
		126.1		Adult on spawning ground
	Salvelinus alpinus	149.7	19	Adult, fresh water
		191.4		Adult, sea water
	Salmo gairdneri	137.2	Houston (unpub.)	Juvenile, fresh water
Potassium	*Salmo trutta*	5.1	11	Fresh water
	Salmo gairdneri	6.13	Spalding (unpub.)	Fresh water
Sodium	*Salmo trutta*	156	11	Fresh water
	Salmo gairdneri	137.3	Spalding (unpub.)	Fresh water
Calcium	*Salmo salar*	3.60	14	Parr
		3.88		Smolt
		4.45		Fresh run adult
		3.36		Adult on spawning ground
		3.27		Adult, downstream migrant

*Duration in sea water not cited.

possibly be attributed to the fact that commonly used open digestion procedures lead to loss of chloride through formation of volatile chloride-containing compounds during the digestion procedure (38). Tissue concentrations of potassium were in good agreement with the values reported for chum, pink, coho, and sockeye salmon (28).

Few estimates of the extracellular volume of fish muscle have been made. Nishie and Harris (33) give values of 123 to 126 g/kg for the muscle chloride space of *Electrophorus electricus*. Data provided by Fontaine, Callamand, and Vibert (14) and Fontaine (12), when recalculated by the simplified method proposed by Conway (7), gave values ranging from 63 to 83 g/kg. The latter estimates are low due to the low values given for tissue chloride concentration.

Recently Thorson (40) has provided values on the extracellular volumes of four species of marine *Chondrichthyes* using inulin as a reference. The data indicated that the extracellular compartments of these species were 106, 118, 132, and 127 g/kg body weight for *Hydrolagus colliei*, *Raja rhina*, *R. binoculata*, and *Squalus acanthias* respectively, and were in reasonable agreement with the values obtained in this investigation.

Premigratory Changes in Water and Electrolyte Metabolism

Plasma and tissue concentrations of chloride, and water, and tissue concentrations of calcium varied inversely with weight. This decrease was most marked for plasma chloride where a decrease of about 20 mmole/l. occurred over the weight range 30 to 40 g. Kubo (25) and Pickford and Atz (34) have indicated that premigratory decreases in chloremia are characteristic of anadromous fish. Fontaine and Callamand (5, 12) have observed this condition in Atlantic salmon and European eels and have suggested that electrolyte depletion plays a role in the initiation of seaward migration by producing an increase in activity levels. Premigratory changes in plasma chloride concentrations may, however, be related to factors other than those suggested by these authors.

The investigations of Hoar (20) and Nishida (32) on the development of "chloride secreting cells" in Pacific salmon suggest that these units play an important role in plasma chloride regulation in the marine phases. In both species studied, *Oncorhynchus masou* and *O. kisutch*, development took place immediately prior to normal migratory periods. It seems likely, therefore, that the development and function of these cells may account for size-related decrease in plasma chloride levels. Alternately, suppression of the chloride absorption mechanism (presumably by hormonal elements) may occur as a preadaptation to marine osmoregulation. Depletion in the latter situation would be primarily due to urinary and diffusion loss rather than to active outward transport. In either case the "demineralization" of pre-migrants may be considered more plausibly to be a correlative of parr–smolt transformation rather than as an initiator of downstream migration.

A correlation may exist between falling chloremias and decreases in the volume of the chloride space. Inorganic electrolytes provide the bulk of the osmotically active material of the extracellular phase. Any loss of electrolyte

from this phase tends to enhance the role of osmotically active but non-diffusible materials such as proteins. Since the latter are predominantly intracellular it follows that loss of inorganic electrolytes from the extracellular compartment tends to increase the contribution of the former to the total tissue osmotic pool. In the maintenance of isomolarity between cellular and extracellular fluids water must then move into the cellular compartment. As a result the volume of this phase increases while that of the extracellular compartment falls.

Adaptation to Sea Water

The adaptation of steelhead trout to sea water was characteristic of most anadromous species inasmuch as both adjustive and regulative responses were observed (3, 15, 23). The adjustive phase was characterized by increased plasma levels of water, chloride, sodium, and potassium. Tissue levels of chloride and calcium rose markedly during this period while that of potassium decreased slightly. On the other hand cellular concentrations of all three electrolytes rose. In part, and particularly in the instance of potassium, enhanced cellular concentrations were a result of a condition of cellular dehydration resulting from the movement of cellular fluids into the extracellular phase.

The duration of the adjustive phase (between 80 and 170 hours) was comparable with the intervals indicated by the work of Black (3) on chum salmon fry (36 hours), of the author on the same species (36 hours, unpublished data), and of Keys (23) on the eel, *Anguilla vulgaris* (approximately 50 hours). Recently Mrs. G. Howells (1959, personal communication) has derived similar values for the adjustive phase in *Salmo gairdneri*, i.e., 40–70 hours, in 50% sea water.

Following the initial adjustive period the fish entered the regulative phase of adaptation during which control was established over plasma and tissue concentrations of electrolytes and over the distribution of water. Over the period of acclimation to sea water, however, these levels did not return to the values established for fresh-water animals, except in the instance of plasma chloride concentrations of certain weight groups. The data tend to substantiate Gordon's conclusion (19) that salmonid fishes regulate blood and tissue concentrations at two distinct levels, one characteristic of the fresh water forms, and the other and generally higher level characteristic of fish in the marine habitat.

(*a*) *Regulation of Chloride*

As chloride ion is predominantly extracellular in position, uptake of chloride from sea water results in a concentration of this compartment. Some chloride was calculated to have entered the cellular phase but this amounted to only about 2% of the total chloride "load" of the fish after 36 hours in sea water (16.1 mmole/kg). Since osmotic inactivation of this ion by complex formation does not readily take place under physiological conditions (27) a tendency for

hyperosmolarity of the extracellular fluids relative to those of the cells must result. Alleviation of this imbalance is correlated with the transfer of water from the latter to the former phase. Restoration of approximate isomolarity is accomplished only with a sharp increase in the volume of the extracellular phase, and with what amounts to a condition of cellular dehydration.

The main mechanisms for chloride homeostasis appear to be active and extrarenal since the urine of marine animals is characteristically low in chloride content (16). Reference has already been made to the ion-transporting cells of the gills. The chloride-secretory mechanism is activated by slight rises in internal chloride concentration (9, 23). Activation times vary from species to species but cytological evidence of secretory activity has been observed within 1/2 to 3 hours after transfer of *Fundulus* into sea water (8, 9), and within 6 hours in *Anguilla* (18). Maximum change was noted in both cases between 15 and 24 hours after transfer into sea water. The primary adjustive and regulative responses to increased chloride levels appear to be, therefore, initial expansion of the extracellular compartment, subsequent extrarenal excretion of chloride ion.

(*b*) *Regulation of Calcium*

The response to calcium uptake was somewhat different since only about 65% of the calcium load of fish in sea water for 26 hours (1.33 mmole/kg) could be accounted for by the calculated expansion of the extracellular phase. The data suggest therefore that a considerable fraction of the calcium taken up in sea water was withdrawn from the extracellular phase through cellular uptake. In the sense that this process reduces the free ion content of this pool by the formation of physiologically and osmotically inactive complexes it may be considered a component, although a passive one, of the homeostatic mechanism. It was of some interest to note that the percentage increases in plasma chloride and calcium concentrations bore an inverse relation to the percentage of load accounted for by the cellular compartment.

While the soft tissues may, by reversible complex formation, withdraw calcium from the extracellular phase, bone probably serves as a more important cation acceptor. The investigations of Rosenthal (36) suggest that bone has the ability to sequester much more calcium than muscle despite the disparity between their relative contributions to body mass.

Calcium taken up by bone has an extremely long biological half life (36). This essentially irreversible combination of calcium with osseous materials has been studied by Neuman and Weikel (31) in mammals. These authors suggest that excess extracellular phase calcium may enter unoccupied lattice positions ("faults") in individual bone crystals. Further intercrystalline exchange, plus overgrowth, may then serve to remove these ions still further from the readily exchangeable bone areas. Thus the calcification of unsaturated bone crystals may also serve as a supplementary regulative process in trout during their adjustive phase of adaptation to sea water.

Summary

(1) The osmoregulatory adaptation of steelhead trout to sea water has been shown to involve an initial adjustive stage during which the animals must tolerate increased plasma and cellular electrolyte levels, and the transfer of cellular fluids into the extracellular compartment.

(2) Adaptation to chloride ion appears to involve an initial expansion of the extracellular space, followed by extrarenal excretion.

(3) Adjustment to raised plasma calcium concentrations was slight. Withdrawal of calcium from the circulating fluids by complex formation with the soft tissues, and possibly by sequestration in bone crystals, appears to operate as a passive process in the regulation of plasma levels of this ion.

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BLASTOCRITHIDIA N.G. (MASTIGOPHORA: PROTOMONADINA) FOR CRITHIDIA (IN PART), WITH A SUBARCTIC RECORD FOR B. GERRIDIS (PATTON)[1]

MARSHALL LAIRD

Abstract

Two quite distinct genera of the family Trypanosomidae Doflein have been confused under the name of *Crithidia* Léger, which, as shown by Wallace some years ago, is properly referable only to certain small, truncate insect flagellates having a stiff flagellum emerging from a funnelled anterior depression, and never at any stage possessing an undulating membrane. However, an error of 50 years' standing still results in the allocation to this genus of arthropod parasites characterized by a paranuclear kinetoplast from which a flexile flagellum proceeds anteriorly along the outer edge of an undulating membrane. It is now proposed that such flagellates be referred to *Blastocrithidia* n.g. The selected genotype, *B. gerridis* (Patton, 1908), a parasite of water striders (Hemiptera: Gerroidea), is recorded from northern Canada for the first time.

There has been much perplexity over the status and interrelationships of certain members of the protomonadinid family Trypanosomidae Doflein. Considerable attention was devoted to insect flagellates early in the century, soon after Léger (6) established the genus *Crithidia*, with *C. fasciculata* of mosquitoes as the genotype. Wallace (15) has clearly demonstrated the validity of this name for a small, truncate mosquito parasite having a stiff flagellum emerging from a funnelled anterior depression, and never at any stage possessing an undulating membrane. Léger was impressed by the organism's resemblance to a tiny barleycorn, the name he chose for it being derived from Greek roots meaning just that. However, his contemporaries, obsessed with the fact that superficially similar stumpy forms occur in the life cycle of insect trypanosomids of other genera, decided that he had mistakenly conferred generic status upon one of these developmental stages. In the making of this claim, great stress was laid upon the fact that Léger, having described *C. fasciculata*, referred to the presence in the same host of longer forms which *may* have had a membrane. Such forms were in fact described under the name of *Trypanosoma culicis* by Novy *et al.* (12), the first to suggest that Léger had dealt with a mixed infection. Patton (13) developed this suggestion along lines hardly in accord with established taxonomic principles—he made the rather astonishing statement that "the young forms of LÉGER'S parasite suggest a young *Herpetomonas* . . . and have none of the appearances of a young *Crithidia*." He proceeded to transfer "*Trypanosoma culicis*" to the latter genus, which was emended as follows: ". . . the adult form is characterised by an attenuated anterior end to which the flagellum is attached by a rudimentary undulating membrane; the blepharoplast in all such flagellates is situated close up to the nucleus, never posterior to it."

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Porter (14), Wenyon (16), and most other protozoologists followed this lead. Where uncertainty was felt as to the real nature of the mosquito parasite described by Léger, this, regardless of the fact that it is the genotype of *Crithidia*, was reclassified without questioning the validity of Patton's emendation. Thus Lwoff and Lwoff (7) established a new subgenus (*Strigomonas*) of *Leptomonas* Kent for its reception, and Grassé (4) considered *Strigomonas* to be of generic rank, with *S. fasciculata* (Léger) as genotype. The misconception that *Crithidia* is characterized by an undulating membrane in advance of the nucleus is so firmly rooted that despite Wallace's elucidation (15), the most recent textbooks in English (5), French (4), and German (2) adhere to Patton's view.

All this would be sufficiently confusing in itself, without the added complication that some developmental stages of *Trypanosoma* Gruby, in the invertebrate host, have the kinetoplast, and usually a short undulating membrane as well, anterior to the nucleus. These are the "crithidia forms" of the very extensive literature on trypanosomiasis. Because of them, several species of *Trypanosoma* initially known only from the invertebrate host were described under the name of *Crithidia* as emended by Patton. Examples are *T. melophagium* (Flu, 1908) of sheep and *T. conorhini* (Donovan, 1909) of rats, originally described as species of *Crithidia* of the vectors, the sheep ked, *Melophagus ovinus* L., and the reduviid bug, *Triatoma rubrofasciata* (de Geer). Minchin (9) contended that although there are undoubtedly valid species of *Crithidia* (Patton emend.) restricted to the gut of certain insects (haematophagous ones not necessarily excepted), "it is safest to presume that any '*Crithidia*' from the digestive tract of a blood-sucking insect is a stage of a trypanosome from the blood of a vertebrate, until the contrary has been clearly established."

Despite the fact that it appeared in a journal of major importance and wide circulation, Wallace's paper has been overlooked by certain authors (3, 10) who have published accounts of new species of *Crithidia* (Patton emend.) during the past decade. Nevertheless, this paper left no doubt as to the status of *Crithidia fasciculata*. It also revealed the embarrassing fact that the crithidia forms of *Trypanosoma* are called after a genus to which they bear little resemblance, and left nameless the species assigned to *Crithidia* in accordance with Patton's emendation. That clarification of the situation is overdue is obvious from these observations, and is emphasized by recent interest in both *Crithidia fasciculata* (11) and "*Crithidia*" derived from phytophagous bugs (8) as subjects for laboratory experimentation—misleading comparisons could easily be drawn as more and more work of this sort is performed by biochemists and others not versed in protozoan systematics.

Blastocrithidia n.g. is accordingly proposed as the generic designation for arthropod flagellates of the family Trypanosomidae in which development never proceeds beyond a stage characterized by a kinetoplast located immediately in advance of, or alongside, the nucleus, a flexile flagellum proceeding anteriorly along the outer edge of an undulating membrane. The

prefix is derived from the Greek *blastos*, and signifies "sprouting," in reference to the appearance of the flagellar apparatus by comparison with that of *Crithidia*. By use of the latter name as the stem, the way remains open to retain the term "crithidia forms" for comparable stages of trypanosomes. In designating the genotype, it must be borne in mind that there is still doubt as to the status of some insufficiently known herpetomonads, leptomonads, and other trypanosomids of insects discovered in the early years of the century. Despite the fact that one or more of these flagellates described from haematophagous and other insects before 1908 may yet prove referable to *Blastocrithidia*, Article 30 of the International Rules of Zoological Nomenclature dictates the selection of *B. gerridis* (Patton, 1908) as the type species.

B. gerridis was first described from Indian species of *Gerris*, *Microvelia*, and *Perittopus* (?) (13). Wenyon (16) summarized various European, African, and North American records. The last-named were those of Becker (1), who found this parasite in the digestive tract of *Gerris remigis* Say, *G. marginatus* Say, *G. rufoscutellatus* Latreille, and *Microvelia americana* (Uhler) in New York and Maryland, U.S.A. The parasite does not seem to have been noted from Canada or from high latitudes in any part of the world.

On June 16, 1959, I collected 10 examples of *Gerris* sp. (aff. *G. marginatus*) from a shallow pond near Kohlmeister Lake (False River), Ungava Bay, Quebec (58° 07′ N., 68° 07′ W.). Great numbers of trypanosomid flagellates were present in the digestive tract of all of these, from the first stomach to the rectum. Giemsa-stained, air-dried films contain posteriorly rounded haptomonad (attached) forms ranging from 6.5 to 26.3 μ in length and from 2.3 to 3.5 μ in breadth. Motile nectomonads in the same preparations include a few short, slender forms having a relatively long free flagellum (accounting for as much as 14.7 μ of an over-all length of 33.1 μ) but mostly comprise individuals having a short free flagellum, a nucleus located one-third of the over-all length from the posterior extremity of the body, and a prominent anterior undulating membrane. Fifty nectomonads (10 consecutive ones from each of five water striders) were measured with the following results (measurements in microns):

	Average for:					
	1st	2nd	3rd	4th	5th	All 50
Posterior extremity to middle of nucleus	15.9	12.4	13.6	11.6	13.0	13.3
Middle of nucleus to kinetoplast	2.1	2.1	2.4	1.8	2.0	2.1
Kinetoplast to tip of free flagellum	26.3	25.4	26.5	22.2	23.2	24.7
Over-all length	44.3	39.9	42.5	35.6	38.2	40.1
Greatest breadth	1.7	1.8	1.5	1.9	1.8	1.7
Length of free flagellum	4.5	6.7	6.2	5.5	4.6	5.5

The range in over-all length was 15.1 to 53.5 μ. Relatively slight differences between these measurements and those of Becker (1) are probably to be accounted for by the fact that his preparations were never-dried haematoxylin ones in which some linear shrinkage is inevitable. There are no grounds for

suspecting that we were not both dealing with *B. gerridis*, agreement with earlier descriptions and illustrations of the various life-history stages (13, 14) being very close indeed.

It is of interest to note that, like Patton (13), I did not find an uninfected water strider—Becker's infection rates for Long Island and Baltimore were 20.5% and 2.5% respectively. In view of current interest in insect flagellates as subjects for biochemical research, it might repay those concerned to investigate local sources of this cosmopolitan species.

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CLIMATE IN RELATION TO WINTER MORTALITY OF THE LODGEPOLE NEEDLE MINER, RECURVARIA STARKI FREE., IN CANADIAN ROCKY MOUNTAIN PARKS[1]

R. W. STARK[2]

Abstract

Four major air masses, tropical maritime (mT), polar maritime (mP), arctic maritime (mA), and arctic continental (cA) may be used in describing the winter climate of a lodgepole needle miner (*Recurvaria starki* Free.) outbreak area. The main circulation is from the north and west which results in a predominance of mP and cA air. Local topography and its effect on winter climate is described for four areas now sampled on a life-table basis. Variations in winter mortality from year to year and between sampling areas are related to climate. Extensive invasions of cA air, particularly of long duration, cause lethal winter temperatures. Winter mortality usually occurs during the coldest month, January, and may be exceptionally high when extreme minima of −30° F to −40° F persist long enough to depress the monthly mean temperature close to 0° F. However, less extreme temperatures in other months may result in similar high mortalities if these are unseasonably low. The climatic conditions causing high mortality generally occur at the tops of slopes and at valley bottoms, allowing the middle slopes to serve as "refuge areas" for surviving needle miner populations.

Introduction

Larvae of the 2-year cycle needle miner in the Canadian Rocky Mountain Parks are exposed to two successive winters during development. It has become increasingly apparent that mortality during these winters has been a major factor in causing population reductions. The relations between the variations of larval mortality and climate are discussed in this paper while the effect that winter mortality has on the epidemiology of the outbreak is discussed in a subsequent publication.

Atmospheric Circulation and the Climate of the Outbreak Area

The climate of a region is determined largely by the types and circulations of air masses. When an air mass remains stationary for some time it acquires properties of temperature and humidity that are characteristic of that region. Air masses can therefore be classified according to these characteristics. The classification that is most commonly used is that of Bergeron, which recognizes four principal source regions: polar (P), arctic (A), tropical (T), and equatorial (E). Air masses are further identified according to their origin over land (c) or water (m). Climatic effects in this area have been described in previous studies by the use of five air masses (1). On the other hand, Penner (3) has shown that, for practical purposes, four major air masses may be used to describe winter weather in North America as follows: tropical maritime (mT), polar maritime (mP), arctic maritime (mA), and arctic continental (cA).

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Penner's system was adopted for the current study and differs from the system used in earlier studies only in that polar continental (cP) is equivalent to arctic continental (cA). The main circulation in the outbreak area is from the north and west, which results in a predominance of mP and cA air. Local topography exerts considerable influence on the behavior of air masses in the outbreak area. The vertical distribution of temperature in the Bow Valley generally is a function of the predominant type of circulation. Thus, in a

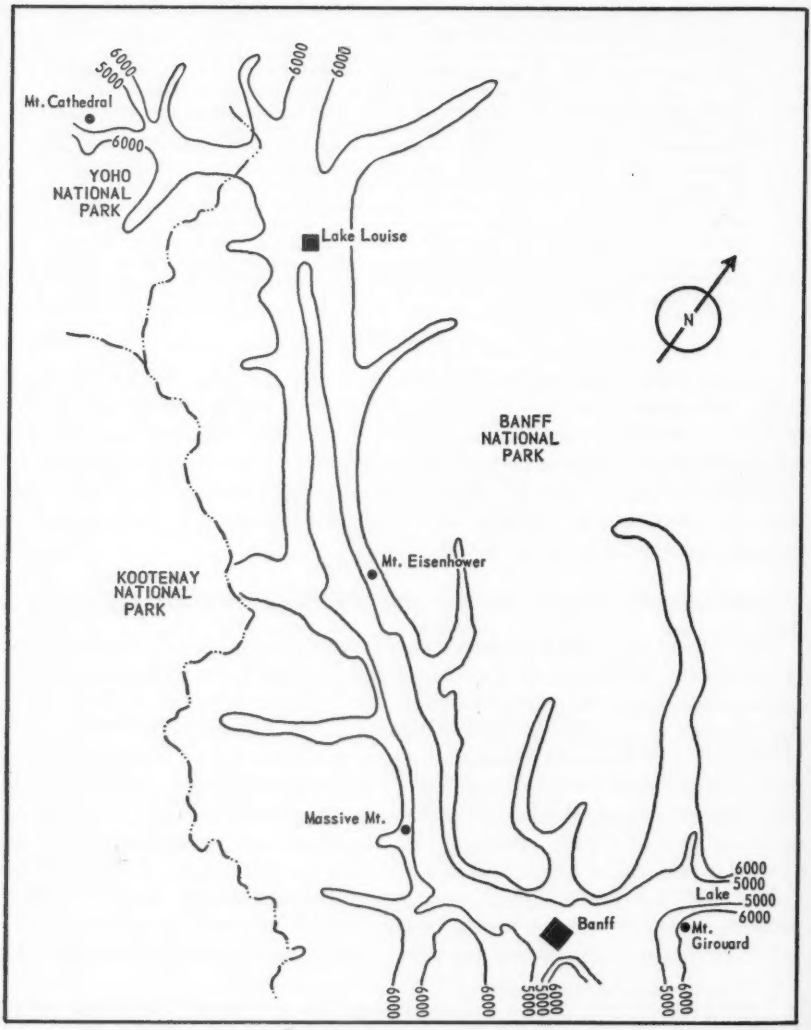


FIG. 1. Schematic illustration of the four sampling areas, the two climatological stations, and the 5000- and 6000-ft contour lines.

month of frequent, rapid invasions of cA air, the upper slopes are usually colder than the valley floor. Extremes of cold at valley bottom are produced when fronts are few and the air stagnates. These conditions are most extreme when the air is of cA origin. The middle of the slopes are consistently warmer than the extreme temperatures whether these occur at valley bottom or the tops of slopes. Considerable variation is found between sampling areas due to local topographic differences such as exposure to trans-divide passes, alpine valleys, and position in the Bow Valley with respect to invading air masses.

The effects of topography on climatic characteristics important to needle miner mortality have been described for four areas (1, 5), three of which are permanent sampling areas. These are redescribed here for the purposes of this study and a description of a fourth permanent sample area, Mount Girouard, added (see Fig. 1).

(1) Massive Mountain.—The slopes of Massive Mountain are protected from the direct influence of trans-divide passes and so are considered representative of most Bow Valley conditions. When cold air invades the valley, the top of Massive Mountain becomes colder than its base while the new air mass is fresh. Approximately equal conditions between the top and base are obtained in a short time but in a winter when cold fronts are plentiful the top of Massive Mountain would be generally colder than its base. On the other hand, if fronts are few, the cold air stagnates and the greatly increased radiational cooling of all surfaces lowers the temperature generally and forms a more and more pronounced inversion in the lower air. Nocturnal temperatures near the valley floor fall much below those at higher elevations and if the air mass persists long enough and is deep, this inversion may persist into the daytime and increase the depth of the cold air pool at valley bottom.

(2) Mount Eisenhower.—This presents a more complicated situation as it is more subject to convective winds and lies directly across the Bow Valley from the mouth of a trans-divide pass, where it is exposed to westerly winds.

(3) Cathedral Mountain.—The climatic conditions are more complex due to the Great Divide, the physiography, and the direction of air flow. Conditions there are obviously not comparable to those in the Bow Valley as the colder cA air is often kept out of the area by the divide or is limited in its western extension. Also, mP air is frequently kept from fully penetrating the Bow Valley.

(4) Mount Girouard.—This area is exposed to air masses coming down the Bow Valley from the northwest and to a limited extent, from the east. It is at the mouth of a large alpine valley which contributes strong down-slope winds and it is also adjacent to a large mountain lake.

Climate and Winter Mortality

Causes of Winter Mortality Variations

Winter mortality is distributed in the same way as zones of extreme cold that occur during the winter. Greatest mortality occurs either at valley bottom or at the tops of slopes, and the middle slopes act as "refuge areas"

for needle miner populations. The low temperatures which are believed to cause winter mortality of needle miner larvae may be produced in somewhat different ways. Cold cA air is the basic requirement but lower temperatures are generally produced when such air remains in the area for some time. Repeated invasions of cold cA air generally do not produce as extreme cold weather as stagnating air but may do so when the air is from a very cold, deep, air mass (1,5).

Extremely high mortality of needle miner larvae occurred in the winter of 1949–50 and it has been demonstrated by comparing this winter with that of 1948–49 that the mean monthly temperatures of 1949–50 were higher than those of 1948–49 with one exception, January. Thus it was concluded that the bulk of winter mortality probably occurred in that month (1). Winter sampling in 1953–54 verified that winter mortality occurred mainly during the month of coldest temperatures, again in January. There is some discrepancy between the mortality estimates in the winter months (particularly February) and the final spring estimate (Table I). This is largely because the condition of larvae examined in February was less easily determined than at the other sampling periods. The great differences between December and February at Mount Eisenhower and Lake Louise and the close agreement between the February and spring samples, for the low elevation at least, leaves little doubt that mortality occurred mainly between December 16 and February 5.

TABLE I

Winter mortality of lodgepole needle miner larvae (1953–54)

Location	Elevation (ft)	Dec. 1953	Feb. 1954	Spring 1954
Mount Eisenhower	4800	4.3%	94.6%	98.4%
	5300	2.4	18.1	17.7
	5800	6.2	15.6	6.3
	6300	—	9.1	1.5
Cathedral Mountain	4500	—	18.4	11.2
	5000	—	31.3	7.0
	5500	3.8	8.7	6.8
Lake Louise	5050	5.4	91.7	99.6
	5550	43.5	11.3	15.8
	6050	13.0	20.6	17.4
	6550	—	8.7	6.8

The maximum and minimum temperatures obtained at Banff and Lake Louise for the period January 10 to 29, 1954, demonstrate the sustained low temperatures that coincided with the heavy mortality which occurred that year (Table II). The Banff – Lake Louise area was in cA air for 22 days in 1953–54, only 3 days fewer than in 1949–50. However, in 1949–50, there were only 3 fronts, 2 of which were cA, whereas in 1953–54 there were 8 cA fronts with a total of 20 of all types. This means that there was less stagnation of cA air in 1953–54 and temperatures throughout the valley had less

chance of becoming as extreme as in 1949–50. These conditions produced temperature inversions with consequent high mortality in valley bottoms. The air-mass producing the temperature inversion would have to be very deep and intense as the stagnation effect is not usual; generally after an air mass is established there is a gradual warming of the air over the area. The longer the air remains in the area, the more marked the difference and the deeper the pool of cold air becomes. A similar reversal of temperature may be produced by a very intense but shallow air mass which warms comparatively quickly. The steep character of the valleys probably contributes to the stagnation effect.

TABLE II

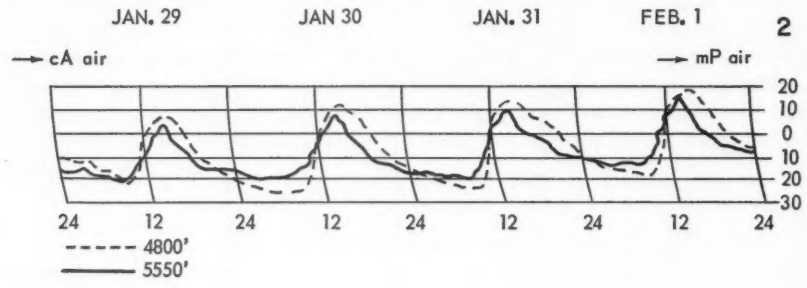
Daily maximum and minimum temperatures (°F), January 10–29, 1954, Banff and Lake Louise

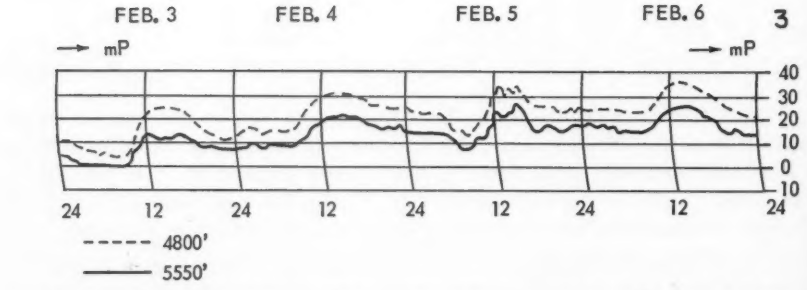
Date	Banff		Lake Louise	
	Max.	Min.	Max.	Min.
January 10	17	− 1	18	−26
11	21	8	26	− 8
12	17	− 5	15	−23
13	18	9	16	7
14	5	−15	− 8	−16
15	−22	−26	−21	−35
16	1	−39	− 3	−52
17	4	−13	− 2	− 8
18	6	−19	8	−31
19	− 9	−18	−15	−32
20	−11	−33	−12	−47
21	−18	−26	−14	−23
22	−16	−23	− 6	−25
23	−15	−25	−14	−22
24	−12	−21	−12	−19
25	− 1	−20	− 7	−20
26	1	−14	15	−12
27	17	−24	14	−21
28	27	10	19	7
29	18	− 3	17	−21

The temperature inversion was demonstrated in 1956 by operating thermographs at two altitude levels, 4800 ft (valley bottom) and 5400 ft (Mount Eisenhower sampling area), from January 2 to February 14. On January 7, after a day and a half of cold cA air there was an inversion from 0700 to 1100 M.S.T. with a peak difference of 12 F degrees. This was due to a weak invasion of mP air, the warming effect of which was felt first on the upper slopes, 5 hours earlier than in valley bottom. The next inversion occurred on January 13. Arctic continental air had invaded the Bow Valley on January 11 but as the circulation was weak, the air mass could not displace the warm mP air completely. This inversion undoubtedly resulted from the cold cA air displacing the warm mP air at valley bottom before it displaced that on the upper slopes. Another short inversion occurred on January 18 when mP air entered the valley, replacing cA air, and in the same day was replaced by cA air again.

This last air mass remained in the valley from January 24 to February 2 becoming increasingly colder until January 30 when a slight moderating trend began. The conditions approached those postulated by Henson *et al.* (1) for 1949–50. The nightly inversion began on January 28 and was repeated each night until February 1.

The total hourly summations from 0100 to 1100 on the last 3 days of the month showed valley bottom to be 59, 53, and 45 degree-hours colder than the upper slopes (Fig. 2). The temperature during this period was not only more severe at valley bottom but was also much more variable. The diurnal ranges at valley bottom were: 9° F to −25° F, 11° F to −23° F, and 13° F to −18° F and on the upper slopes: 5° F to −19° F, 8° F to −19° F, and 10° F to −13° F. Such conditions are presumed to account for the observed mortality differences and for the confining of high mortality to valley bottom. The typical temperature–altitude relationships are illustrated in Fig. 3 for the same area when mP air had replaced the cA air.





FIGS. 2 and 3. Temperature conditions under arctic continental air (Fig. 2) and under polar maritime air (Fig. 3).

Winter Mortality of Needle Miner Populations

Yearly estimates of winter mortality since 1944 demonstrate the importance of low temperatures as a control factor (Table III). The estimates are based on 86 samplings from 23 areas (5), mainly from the 4 areas described above.

The accuracy of the estimates prior to 1948 is unknown, since that time it is within 10% of the mean. It was concluded from these and other data that five winters since 1943 caused high larval mortality, as follows:

Winter of 1945–46.—Mortality was apparently high, although the degree of mortality is not certain. Daily temperatures for this winter at Banff and Lake Louise show that, in general, it was mild but extremely variable. Thus a minimum of −35° F at Lake Louise and −26° F at Banff was recorded on November 8, whereas on November 3 the minimum was 31° F. Similar, but less extreme cases, occurred throughout the winter. Air-mass analyses show that in November the area was in cA air for 12 days (5). This amount has been exceeded only once (1927–28) and equalled once (1935–36) since 1920.

TABLE III

Winter mortality of lodgepole needle miner larvae (all areas)

	Valley bottom (av. 4800 ft)		Elevation above valley bottom					
			Up to 500 ft		500–1000 ft		Over 1000 ft	
Year	% mort.	N*	% mort.	N	% mort.	N	% mort.	N
1943–44	—		9.0	1	—		—	
1944–45	—		—		—		—	
1945–46	—	Approx.	50.0	1	—		—	
1946–47	—		1.0	1	—		—	
1947–48	—		20.0	1	—		—	
1948–49	24.6	5	19.4	5	13.7	5	23.6	5
1949–50	97.7	5	82.8	5	74.9	5	75.7	5
1950–51	—		25.0	1	—		—	
1951–52	—		11.3	2	12.6	2	—	
1952–53	5.6	2	5.6	1	—		—	
1953–54	92.2	4	12.2	5	6.5	4	3.7	3
1954–55	89.7	2	65.9	6	73.0	6	—	
1955–56	100	1	27.3	2	23.7	1	—	

*N = Number of areas sampled at each altitude.

Winter of 1949–50.—Earlier discussion of the 1949–50 winter climate showed that the weather of the coldest month, January, was the major cause of needle miner mortality for that period (1).

Winter of 1953–54.—The high mortality which occurred in this winter almost certainly was due to the temperatures which obtained during January, 1954 (Table I). November and December were the mildest during the period 1945 to 1956 but the January mean temperature was lower than the winter of low mortality in 1948–49. The mild November and December may have decreased the resistance of the needle miner larvae to the low temperatures of January (4,6). The mean minimum was higher than that of 1948–49 but the mean maximum was lower. The maximum was below zero for 8 days and the minimum for 18 days in 1953–54, whereas in 1948–49 the maximum was below zero for only 4 days and the minimum for 17. Also, in 1953–54 the maximum temperature was below zero for 7 consecutive days and the minimum for 14 consecutive days, whereas in 1948–49 the maximum was below

zero for only 2 consecutive days and the minimum for only 8 consecutive days. The air mass analyses show that January, 1954, had 22 days of cold cA air, whereas January, 1949, had only 15 days of cA air. Variations in high mortality are compatible with these data. The conditions of this winter were intermediate between the low mortality year, 1948–49, and the high mortality year, 1949–50. The high mortality in 1953–54 was restricted to valley bottom in contrast to 1949–50 when mortality was high at all altitudes.

Winter of 1954–55.—The observed mortality was similar to, although less severe, than that of 1949–50. Temperature conditions presumed responsible for this are less clear-cut than those in 1949–50. Frontal activity was reduced and there were fewer days in which the area was in cA air. The high mortality probably occurred in March, when cA air persisted in the Bow Valley for 16 days, which has been exceeded only three times and equalled twice since 1920. This was the coldest March since 1945 with the exception of 1951 which was a year of low mortality. There were three periods of below-zero minima separated by above-freezing maxima in 1955, whereas the cold period responsible for lower means in 1951 was restricted to the beginning of the month.

Winter of 1955–56.—Mortality was confined largely to valley bottom and was comparable to that of 1953–54. Monthly mean temperatures were not unusually low but there was an extremely cold period from February 18 to March 5 and again from March 19 to March 26. It is assumed that mortality occurred mainly during these periods.

Discussion

A comparison of the winter temperatures for years of high mortality with those for years of low mortality gives some indication of the low temperature tolerance of the needle miner. It is concluded from the above data and previous studies (1,5) that, in general, the lodgepole needle miner population in the outbreak region of Banff Park can have a high survival if extreme minima of $-30°$ F to $-40°$ F do not persist long enough to depress the mean monthly temperature close to or below the zero mark. This generalization must be considered both as to the time of year in which low temperatures occur and to the duration of cold cA air, that is, such extremes of cold would probably not be necessary to cause high mortality in November or March. It is believed that mortality usually occurs during the coldest month, January, but may occur occasionally with equal intensity in any other winter month. The assessment of critical periods requires more than an examination of extreme minima, mean maxima and minima, and mean monthly temperatures because when cold periods develop which overlap into two months their effect is not reflected in means and a careful scrutiny of daily temperatures is necessary.

Fluctuations in needle miner populations in Yoho and Kootenay Parks are subject to speculation only because temperature data and detailed knowledge of air-mass movements are lacking for these areas. The populations in these areas have subsided at equivalent rates to, but for different reasons than those in Banff Park. Climatic factors other than low winter temperatures are

probably the cause as neither parasites nor disease have figured prominently in the control complex. Conditions in Yoho Park are more variable, though less extreme, than in Banff Park, and while these conditions have not resulted in excessive mortality except in later years (5) it is possible that they have affected the population in other ways, perhaps through development, fertility, and/or fecundity. The pattern of mortality in Kootenay Park approximated that for Banff. Altitudinal and physiographic differences between the two parks are probably influential in causing any variations noted.

Increased use and refinement of air mass and frontal analysis techniques and knowledge of local variations of climate in the Bow Valley already discussed permit the development of a predictive system for use in population studies. For example, a successful prediction was made that winter mortality of the 1956–58 generation of needle miner for both winters would be low and that of 1957–58 lower than that of 1956–57. It is now reasonably certain that the persistent infestations will be centered on the mid-slopes in the Bow Valley of Banff National Park. Although populations may increase above and below this "refuge" zone for a few years, they will eventually suffer high mortality in years of severe winters (1,5).

Acknowledgments

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A CONTRIBUTION TO THE BIONOMICS OF THE MOSQUITOES OF ALGONQUIN PARK[1]

W. E. Beckel and H. L. Atwood

Abstract

The results of one season's mosquito collecting in Algonquin Park are presented giving data on relative abundance, seasonal distribution, and habitat of larvae and adults.

General correlations between mosquito distribution and vegetation, and distribution and exposure are mentioned.

The list of species found in the Park now includes 3 species of *Anopheles*, 17 of *Aedes*, 3 of *Culiseta*, 2 of *Culex*, 1 of *Wyeomia*, and 1 of *Mansonia*. Twenty-one of these 27 species have been taken biting man. Of these the most common in order of time of peak abundance were *Aedes communis*, *Aedes punctor*, *Mansonia perturbans*, and *Aedes canadensis*.

Much interest has been shown in the bionomics of the mosquitoes of Canada (2) but no extensive information exists for the mosquitoes of Ontario. This paper presents the data from one season's study of the mosquitoes in Algonquin Park. Such data based on one season's results are usually inadequate for generalizations about any area but we present our results to aid any further research on mosquitoes in the park or in other parts of Ontario.

Description of the Terrain Studied

Algonquin Park lies in the southern edge of the Canadian Shield, a complex series of Pre-Cambrian granite rocks overlaid by glacial deposits. In the Park these deposits are mostly of sand or gravel molded into many small hills and valleys. The park lies in the "Great Lakes – St. Lawrence" forest region (1), in which the most significant tree species are hard maple (*Acer saccharum*), white birch (*Betula papyrifera*), yellow birch (*Betula lutea*), black spruce (*Picea mariana*), balsam fir (*Abies balsamea*), white pine (*Pinus strobus*), and red pine (*Pinus resinosa*).

The main areas in the park visited by us during the summer are shown in Fig. 1.

Fig. 1. A map of the region of Algonquin Park where the study was made. A1–A4, shown by a broken line, are the major areas visited.

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Contribution from the Department of Zoology, University of Toronto, Toronto, Ontario.

Area 1—South End of Lake Opeongo

This area was made up of numerous hills, stream valleys, and a variety of marshes, bogs, meadows, and different forest types. The forest, on mesic sites, was usually composed either of hard maple or of a mixed forest of red pine, white pine, and white birch. In lowland areas the dominant tree species was black spruce. Most of the information to be presented on larval collections was obtained from area 1.

Area 2—Lake Sasejewun

The area visited lay in the valley of the Madawaska River. The bottom of this valley was flat, sandy, and bounded by abrupt hills on either side. The forest consisted mainly of white pine, white birch, balsam fir, and hard maple.

Area 3—South Tea Lake

Several hills, bounding the lake and covered with a mature forest of hard maple, were visited.

Area 4—Opeongo River

The valley of this river, below Booth Lake, was flat in many places close to the water. This gave rise to low-lying marshes which were flooded by high water. The forest near the river was largely poplar, indicative of relatively recent forest fire. The soil was sandy.

Methods

The field work was started on May 18 and ended September 8. During May and June collections of larvae and pupae were made from as many sites as possible. As area 1 was the most accessible, the largest number of collections were made here.

Data were taken on vegetation and exposure to sunlight at each site. The larval and pupal collections were numbered and brought to the laboratory where the larvae were reared individually in 4-oz bottles filled with Opeongo Lake water to which a small amount of commercial dog food was added to support growth of bacteria. These larvae could be accurately identified. The adults emerging from such rearings were stored with the last-cast larval skin to ensure positive identification. Pupae collected in the field, when larvae were no longer available, were reared in water without food. Many adults emerged from these field-collected pupae; identification of these adults was less certain in the absence of associated larval skins, but was considered more certain than identification of field-collected adults.

Field collections of adults were made in many locations during the summer. Positive identifications of all these specimens was sometimes difficult even with the present taxonomic keys. Such collections were valuable because several species were collected which had not been obtained from larval and pupal collections, and because information concerning approximate time of appearance in the field and distribution of adult mosquitoes was obtained from them.

Adult mosquitoes were collected every day throughout the summer in area 1. Collections were made in area 2 at least once a week; area 3 was visited twice in July; area 4 was visited once in mid-July, once in late July, once in early August, and once in early September. At each visit to an area, collecting was done throughout the day in different habitats by sitting quietly in each habitat for at least ½ hour and putting a killing bottle over mosquitoes settling on the clothes or exposed skin of the collector or of a colleague. Also sweeping of the vegetation around larval habitats yielded many mosquitoes not attracted to man.

Summary of the Weather (May 18 to September 8) and Associated Data

From May 18 to June 24 the weather was mainly sunny and warm. There was rain on 11 of these 38 days but it was mostly in the form of light showers or thunderstorms. Temporary pools left by the melting of the snow in early May dried up during June.

A very heavy rain (2 to 3 in. of rainfall) occurred from June 25 to June 30 as a result of Hurricane Audrey. This rain filled the temporary pools again and raised the water level in lakes, bogs, and marshes in most cases to a level higher than the spring high-water mark. This is an atypical condition for late June and consequently appears to have had an atypical effect on the larval density. Just before the rain the larval density of most species of *Aedes* was very low. After the rain an unusual second hatch of certain *Aedes* species occurred. The main species involved were *Aedes punctor*, *A. canadensis*, *A. communis*, *A. dianteus*, and *A. cinereus*.

As a rough phenological guide to the early part of the season the following are the dates on which some of the common plants were in flower in area 1:

May 18—*Myrica gale*, *Lonicera canadensis*.
20—*Viola blanda*, *Fragaria virginiana*.
25—*Prunus pennsylvanica*, *Tiarella cordifolia*, *Andromeda glancophyllum*.

June 1—*Smilacena trifolium*, *Kalmia polifolia*, *Calla palustris*, *Prunus virginiana*.
5—*Acer pennsylvanicum*, *Trillium undulatum*.
8—*Hieracium aurantiacum*.

Results

Larval habitats in area 1 where most of the larvae were collected are described in Table I. Table II presents the mosquito species found as larvae, their habitats, and their relative abundance with time. In contrast to the species of *Aedes*, the larvae of *Culiseta* and *Culex* were found from early June until September. These species are known to have more than one generation a year.

TABLE I

Description of larval habitats in Algonquin Park, area 1

Type	Drainage	Surface dominant	Vegetation, other	Erect dominant	Vegetation, other	Exposure	Remarks
A (heath bog) 4*	Poor: water-level close to surface or above	*Sphagnum* spp.	*Vaccinium oxycoccos*, sometimes *Sarracenia purpurea*	*Chamaedaphne* sp., *Ledum* sp., *Myrica gale*	*Andromeda* sp., *Carex paupercula*, *Carex* spp., *Eriophorum* spp., a few small *Picea mariana and Larix laricina*	Mostly exposed to the sun	Water brown and acid; pools present throughout the summer
B (alder bog) 2	Poor; water-level close to surface	*Sphagnum* spp.	*Vaccinium oxycoccos*, *Smilacena trifolium*	*Alnus* sp. (up to 10 ft high), *Picea mariana* (up to 15 ft high), not a dense stand, *Spirea alba*	*Ledum* sp., *Chamaedaphne* sp., *Scirpus cyperinus*, *Agrostis alba* in places	Variable; usually partly shaded	Water brown and acid; pools present throughout the summer
C (bog forest) 5	Poor; water-level close to surface	Often absent, leaving floor of spruce needles	*Sphagnum* spp., sometimes present; *Carex trisperma*	*Picea mariana* in dense stands up to 40 ft high	*Abies balsamea*, *Ledum* sp., *Vaccinium angustifolium*	Well shaded	Water brown and acid; pools numerous early in year, many dry by July
D (alder swamp) 8	Low-lying but usually partly drained	Often absent leaving floor of leaves	Some mosses (*Sphagnum* spp., *Mnium* spp.) sometimes present	*Alnus* sp. forming dense cover	*Picea mariana*, *Abies balsamea*, *Betulas* spp., grasses and sedges occasional	Well shaded except in May before leaves are out	Pools located in depressions, mostly temporary
E (deciduous forest) 4	Good	Usually absent leaving a floor of leaves	*Viola* spp., *Coptis groenlandicus*	Variable, *Acer* spp., *Betula* spp.	*Pinus* spp., *Abies balsamea*	Well shaded	Small temporary pools in depressions in the forest floor, often formed by melting snow
F (meadows) 7	Usually poor, water levels close to surface	Often *Sphagnum* spp.	Other mosses	Grasses and sedges, typically *Scirpus cyperinus* and *Agrostis alba*	*Spirea alba*	Exposed or poorly shaded by low vegetation	Beaver meadows and grassy areas beside ponds and creeks; most pools present until late July
G (gravel pit) 3	Good	Absent	Absent	Absent	Absent	Exposed	Temporary pools in sand or gravel pits; usually small
H (roadside ditch) 2	Poor	*Sphagnum* spp.		*Alnus* spp., *Picea mariana*	*Salix* spp., *Chamaedaphne* sp., *Glyceria canadensis*, *Agrostis alba*, *Carex* spp.	Partly exposed	Roadside ditches with little flow; bog forest on one side, gravel road on other, water present all summer
I (permanent pool) 1		Absent	Absent	*Juncus brevicaudatus*, *Glyceria canadensis*, *Scirpus cyperinus*	Other grasses and sedges	Exposed, nearby alders and poplars giving some shade	Pool in low-lying sandy depression 20 sq. yd in area; extensive shallow margins, water present until late August

*The numbers indicate the number of samples on which the description is based.

TABLE II

Distribution of mosquito larvae per habitat and time in Algonquin Park, area 1

Species	Habitat type									Time of year			Remarks
	A	B	C	D	E	F	G	H	I	First seen	Most seen	Last seen	
Anopheles earlei									p	e–Jy		e–A	In groups among floating vegetation
A. punctipennis									r	e–Jy			Only a few larvae found
Aedes abserratus	*c	*c	r	d		c		p		m–M		l–J	Larvae associated with *Aedes punctor* but not as numerous, no late hatch seen in July
A. canadensis	p	*c	*c	*c	c	p	p	p		m–M	(1)e–J (2)e–Jy		Extensive late hatch after rain at end of June
A. cinereus	r	*c	r	p		*c	r	r		m–M	e–J	m–Jy	Very small late hatch in early July
A. communis	r	c	c	c	c	c		p		m–M	l–M	m–Jy	Limited late hatch in early July
A. decticus		r									J		Intensive sampling revealed only a few larvae
A. dianteus		p	*c	r	r	r		r		m–M	e–J	M–Jy	Late hatch in common habitat in early July
A. excrucians	r	p		p		*c	r	c		m–M	e–J	m–J	No late hatch
A. fitchii						r				m–M		e–J	Associated with *A. excrucians, punctor, cinereus*
A. intrudens		r				r				m–M		e–J	Rare in area 1
A. pionips		r			r					m–M		m–J	Very rare
A. punctor	*c	c	*c	*c	p	c	r	p		m–M	(1)e–J (2)e–Jy	l–Jy	Very abundant, extensive late hatch in July
A. trichurus		r				r				m–M		e–J	Very rare
Culex restuans			*c	c		r				l–J	Jy	l–A	Common in shaded, stagnant pools
Culex territans	*c	*c	r	r		*c			c	m–M	l–Jy	l–A	Common in open exposed pools
Culiseta morsitans	*c	c				c			p	m–M	J	l–Jy	In exposed pools
Culiseta impatiens			*p	r		r				e–J	Jy	l–A	Often associated with *Culex restuans*
Wyeomia smithii	p									e–J		l–A	In leaves of pitcher plants

(ABBREVIATIONS: c, common; p, present not rare; r, present rare; *found most often; e, early; m, mid, l, late; M, May; J, June; Jy, July; A, August; S, September)

TABLE III

List of species of all stages found in Algonquin Park with data on the adults of these mosquitoes from observations made in the field
(For meanings of abbreviations see Table II)

Species	Area				Time of year			Circumstances of collection	Remarks
	1	2	3	4	First seen	Most seen	Last seen		
Anopheles earlei	r	r			l–J		A	Some biting man, some swept from grasses	
A. punctipennis		r			e–S			Biting man, evening	Only 2 adults taken
A. walkeri	p	p			l–J	e–S	m–S	Biting man, evening	Taken near marshes, rare until September
Aedes abserratus	p	p						Biting man	See *Aedes punctor*
A. aurifer	p				l–J	m–J	e–A	Biting man, day or night mostly in early evening	Found in grassy areas near large open pools
A. canadensis	c	p	p	p	l–J	l–Jy	A	Biting man, day or night	Most abundant biter in late July
A. cinereus	p	p	c	c	m–J	l–Jy, e–A	m–A	Biting man, day or in the evening	Ofter found in grassy areas near the water
A. communis	c	p	p	p	l–M	e–J	m–A	Biting man, day or night	Widely distributed
A. decticus	r	r			l–J	e–J	m–A	Biting man in shade on calm afternoons	Localized around type C habitats
A. dianteus	p	p			l–J	m–Jy	l–A	Biting man in shade	Common in type C habitats
A. excrucians	p				l–Jy		e–S	Biting man	Widely distributed in area 1
A. fitchii	r	r		p	e–Jy		l–A	Biting	
A. implicatus								Not collected, one specimen reported	Reared from pupa in 1956 by other workers
A. intrudens	r	p		p	m–J		e–A	Biting man in buildings by night, outside by day	
A. pionips	r				m–Jy		m–A	Biting man	Very rare
A. punctor	c	c	p	p	l–M	l–J	l–M	Biting man day or night	Widely distributed
A. sticticus				c	m–Jy		S	Biting man day or night, readily enters buildings	Found near areas where flood plains prevalent
A. trichurus								Not collected as adult	
A. triseriatus	r		p		m–Jy		l–Jy	Biting man in shade during the day	Associated with hardwood hills
A. vexans	r	r		p	e–Jy		e–A	Biting man day, early evening	
Culex restuans	p							None taken biting man	Collected from grass around breeding sites
Culex territans	p				l–Jy		e–A	Same as *restuans*	
Culiseta impatiens	r				m–M		l–M	Biting man in buildings	None taken biting except in May
Culiseta inornata		r						Light trap	In 1956 by other workers
Culiseta morsitans	p				m–Jy		l–A	Same as *restuans*	
Mansonia perturbans	c	c	p	p	l–J	Jy	e–S	Biting man, especially at night	Major pest in July
Wyeomia smithii								None collected	

Certain generalizations can be made between larval distribution and vegetation. Some of the species could tolerate a much broader spectrum of habitats than others; for example, *Aedes communis* could be found in almost any small body of water in late May, and the same was true of *A. punctor* in mid-June. In species which had a wide range of habitat, living in both densely shaded and exposed pools, larvae reached maturity much earlier in the more exposed habitats. Thus adult emergence in these cases was spread over an extended period of time.

Many species showed preference for either exposed or shaded habitats. Some species, such as *Culex territans* and *Culiseta morsitans*, were found mainly in exposed habitats, such as bogs and pools in meadows. Others, such as *Culiseta impatiens*, were confined to deeply shaded habitats such as black spruce forest pools.

There was some correlation of larval distribution with vegetation when exposure was the same; for instance, certain species such as *Aedes fitchii* and *A. cinereus*, which were found in pools in grassy meadows, were rare or absent in bogs.

Table III lists all the species found to date in Algonquin Park with data on the occurrence of the adults per area, the relative abundance with time, and remarks including how and where the adults were collected. The field collections of adults revealed marked differences in the mosquito faunas of different areas of the park. Even areas fairly close together often had different species present. Some of these differences could be readily correlated with habitat. For instance, *Aedes triseriatus*, which develops in tree holes, was common at South Tea Lake, where there was a mature deciduous forest, but was rare at Lake Opeongo where there was little such mature forest and consequently fewer development sites. Another example involves two species which are primarily flood-water species, *Aedes vexans* and *A. sticticus*. These two species were common along the Opeongo River, where there were extensive marshes along low-lying river banks. They were almost totally absent from Lake Opeongo where there was no comparable habitat.

There was no obvious explanation why *Aedes intrudens* was found commonly in area 2 around Lake Sasejewun, rarely in area 3 around South Tea Lake, and not at all in area 1 around Lake Opeongo.

Figure 2 shows the relative abundance in area 1 of the most common biting species, as adults, with time. The ratio of the heights of the curves at any one common point represents the ratio of the populations on that day as judged by collections from a number of standard habitats. The collecting consisted of remaining quiet in one place and taking (in a killing bottle) all the mosquitoes on the collector's forearm during a $\frac{1}{2}$-hour period of time. The first species to become abundant was *Aedes communis* in late May and June; it was followed by *Aedes punctor* during June and July. *Mansonia perturbans* followed by *Aedes canadensis* was the most numerous biter in July and August. In certain habitats other species were occasionally abundant as biters; for example *Aedes dianteus* in bog forests and *A. aurifer* in certain meadows.

The mosquito fauna of the park has some characteristics of the fauna of southern Ontario as well as that of northern forest and forest transition tundra regions. *Aedes trichurus*, which is common in southern Ontario, appears rarely in the park; and such species as *A. pionips*, *A. punctor*, and *A. communis* occur north to the tundra.

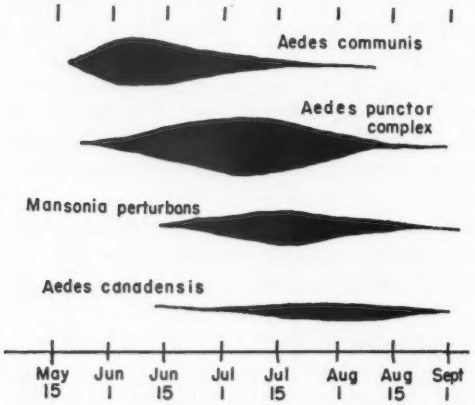

FIG. 2. The relative abundance of the major pest mosquitoes in Algonquin Park. The ratio of the heights of the curves at any one common point represents the ratio of the populations on that day as judged by collections from a number of standard habitats.

Acknowledgments

The authors are grateful for the co-operation of Mr. N. V. Martin, Officer in Charge, Department of Lands and Forests Research Station on Lake Opeongo. We also wish to thank Mr. William Young for assistance in rearing and collecting. Of considerable importance to this work was the financial assistance from the Canadian National Sportsmen Show (through the Toronto Anglers and Hunters Association) and the Ontario Research Foundation.

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NEMATODE PARASITES OF VERTEBRATES OF EAST PAKISTAN

III. CAMALLANIDAE FROM FISH, AMPHIBIA, AND REPTILES[1]

S. P. GUPTA[2]

Abstract

Three species of Camallanidae Railliet and Henry, 1915, are described, namely, *Camallanus nodulosus* sp. nov., from *Rana cyanophlyctis*, *C. thapari* sp. nov., from *R. tigrina*, and *Procamallanus daccai* sp. nov., from an unidentified catfish. In addition *Camallanides* sp. from *Hydrophis cyanocindus* is described. A key to species of the genus *Camallanus* Railliet and Henry, 1915 from amphibia is given.

Nine species of the genus *Camallanus* Railliet and Henry, 1915 have been described from amphibia, namely, *Camallanus microcephalus* (Dujardin, 1846) Railliet and Henry, 1915 (13), *C. nigrescens* (von Linstow, 1906) Railliet and Henry, 1915 (13), *C. baylisi* Karve, 1930 (5), *C. multiruga* Walton, 1932 (15), *C. pipientis* Walton, 1935 (16), *C. kaapstaadi* Southwell and Kirshner, 1937 (14), *C. mazabukae* Kung, 1948 (10), *C. multilineatus* Kung, 1948(10), and *C. ranae* Khera, 1954 (7).

Camallanus nodulosus

(Figs. 1–5)

The present species is represented by three females and three males recovered from the small intestine of *Rana cyanophlyctis* from Dacca, East Pakistan.

Length: male 7.9 to 11.1 mm, female 21.6 to 23.35 mm; width: male 0.22 to 0.3 mm, female 0.4 to 0.5 mm. Cuticle thin, finely striated. Mouth surrounded by two lateral and four submedian papillae. Dorsoventral diameter of head 0.1 to 0.12 mm in male, 0.15 to 0.17 mm in female. Valves of buccal capsule broader than long, 0.08 to 0.09 mm long, 0.09 to 0.11 mm in maximum breadth in male; 0.11 to 0.13 mm long, 0.13 to 0.18 mm wide in female. Buccal valves have 9 to 11 beaded chitinous ridges (10 to 11 in female, 9 to 11 in male (Fig. 1)). Large, stout chitinous ring at junction of valves and oesophagus, 0.06 to 0.085 mm diameter in male, 0.09 mm in female. Tridents (Fig. 2) well developed except for middle prong which is small, and measures 0.03 to 0.04 mm in length in male, 0.027 to 0.042 mm in female. Lateral prongs 0.04 to 0.06 mm long in male, 0.07 to 0.09 mm in female.

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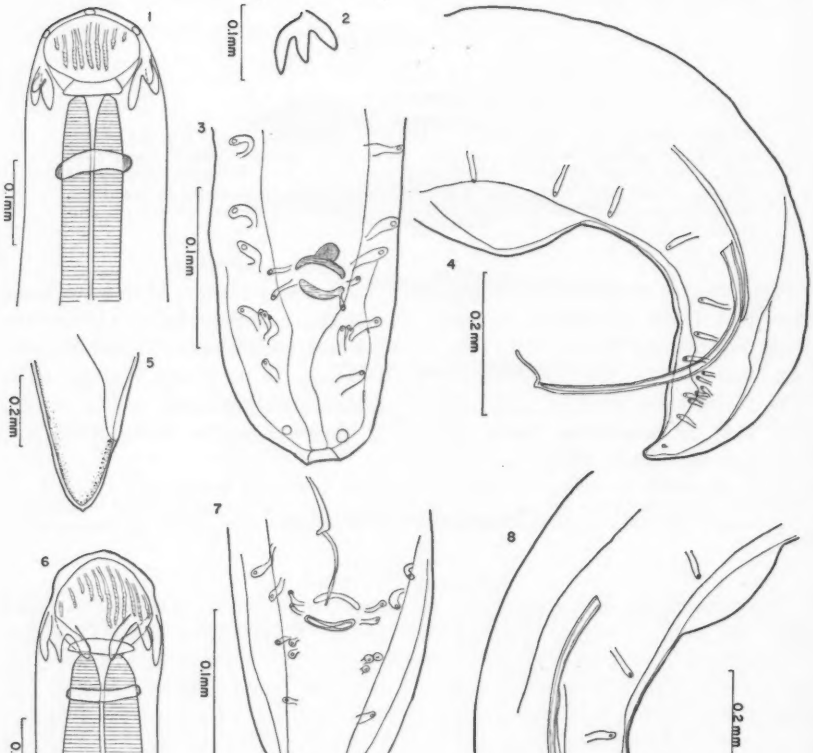
Contribution from the Institute of Parasitology, McGill University, Macdonald College P.O., Que., Canada. The parasites upon which this paper is based were collected by Dr. Robert E. Kuntz (Cdr. MSC., USN; U.S. Naval Medical Research Unit No. 2, Taipei, Taiwan), a member of the U.S. Naval Medical Mission to East Pakistan in 1958. He was assisted by James Reese, HM, IC, USN, and Charles Knight, HCM, USN, also of NAMRU-2. Dr. Robert F. Inger, Curator of Reptiles, Chicago Natural History Museum, has provided the identifications of the fish, amphibia, and reptiles.
[2]National Research Council of Canada, Postdoctorate Fellow.



FIGS. 1–5. *Camallanus nodulosus* sp. nov. Fig. 1. Anterior region of male. Lateral view. Fig. 2. Trident. Fig. 3. Male tail, ventral view. Fig. 4. Male tail, lateral view. Fig. 5. Female tail, lateral view.

FIGS. 6–9. *Camallanus thapari* sp. nov. Fig. 6. Anterior region of male, lateral view. Fig. 7. Male tail, ventral view. Fig. 8. Male tail, lateral view. Fig. 9. Female tail, lateral view.

Anterior club-shaped muscular portion of oesophagus 0.39 to 0.47 mm long in male, 0.64 to 0.69 mm in female; width in male 0.09 to 0.1 mm, in female 0.11 to 0.18 mm. Posterior cylindrical glandular portion of oesophagus 0.34 to 0.4 mm long in male, 0.46 to 0.56 mm in female, 0.09 to 0.11 mm wide in male, 0.1 to 0.11 mm in female.

Nerve ring situated 0.15 to 0.18 mm (Fig. 1) from anterior end in male, 0.175 mm in female. Excretory pore 0.42 mm from anterior end in female.

Male (Fig. 3).—Tail 0.12 to 0.13 mm long with bifid tip. Caudal alae (Fig. 4) long and narrow, beginning at cuticular swelling on ventral side of body, 0.63 mm from posterior end and extending to tip of tail. Seven pairs precloacal papillae at approximately regular intervals except for posterior two pairs which are close together near the cloaca. Two pairs small, pedunculated paracloacal papillae. Six pairs postcloacal papillae, the two posterior pairs of which are isolated, the fifth pedunculate, and the sixth sessile. First four pairs of postcloacal papillae close together forming a group on each side. Two subequal spicules, the right relatively stout, 0.44 to 0.45 mm long with a barb or prong about 0.05 mm long near the tip (Fig. 4). Slender left spicule, 0.27 to 0.36 mm long.

Female.—Tail (Fig. 5) 0.1 to 0.19 mm long, rounded with two small cuticular processes at its extremity. Vulva anterior to mid-body, 9.6 to 10.05 mm from anterior end, a distinct lobe-like structure forming anterior lip.

C. nodulosus sp. nov. differs from all known species of the genus *Camallanus* Railliet and Henry, 1915 described from amphibia and reptilia in possessing beaded or noded ridges (Fig. 1) in the buccal capsule. Only three species (*C. anabantis* Pearse, 1933 (12), *C. sweeti* Moorthy, 1937 (11), and *C. unispiculus* Khera, 1954 (7)) described from fishes possess noded or beaded longitudinal ridges in each valve. However, *C. nodulosus* differs from these three species in the possession of fewer pre- and post-cloacal papillae.

C. nodulosus closely resembles *C. ranae* from *Rana cyanophlyctis* Khera, 1954 (7) and *C. baylisi* of *R. tigrina* Karve, 1930 (5) from India. However, *C. nodulosus* differs from *C. ranae* in having six pairs of postcloacal papillae (Fig. 3) instead of the five pairs present in *C. ranae*. *C. nodulosus* possesses a barb or prong (Fig. 4) about 0.05 mm long at the tip of the left spicule and has two cuticular processes at the tip of the female tail.

C. nodulosus can be distinguished from *C. baylisi* in the arrangement of the postcloacal papillae. In *C. baylisi* the most posterior are close together in a group while in my species the most posterior postcloacal papilla (Fig. 3) is sessile and the four anterior posterior anal pedunculated papillae are close together in a group. *C. nodulosus* differs also from *C. baylisi* in having a much larger left spicule; the right spicule in both species is approximately the same size. The left spicule in *C. baylisi* measures 0.15 to 0.23 mm, while in *C. nodulosus* it is from 0.27 to 0.36 mm long.

Host.—Rana cyanophylectis.

Habitat.—Small intestine.

Locality.—Dacca, East Pakistan.

Camallanus thapari sp. nov.

(Figs. 6–9)

The material consisted of 11 specimens, 5 females and 6 males, recovered from the small intestine of *Rana tigrina* from Dacca, East Pakistan.

Male 9.27 to 13.1 mm long, female 20.2 to 27.9 mm. Male 0.25 to 0.29 mm wide, female 0.45 to 0.6 mm. Cuticle thin, finely striated. Mouth surrounded by two lateral and four submedian papillae. Dorsoventral diameter of head in male 0.1 to 0.13 mm, 0.18 to 0.2 mm in female. Buccal capsule formed by two buccal valves which measure (excluding posterior ring) 0.076 to 0.9 mm long, and 0.1 to 0.11 mm wide in male, 0.12 to 0.15 mm long, 0.15 to 0.16 mm wide in female. Male buccal valves possess 9 to 11 ridges (Fig. 6), female, 11 ridges. Large stout chitinous ring (0.075 to 0.08 mm in diameter in male, 0.09 to 0.1 mm in female) present at junction of valves and oesophagus. Tridents well developed, the middle prong measuring 0.04 to 0.048 mm in male, 0.035 to 0.05 mm in female; lateral prong 0.05 to 0.06 mm in male, 0.05 to 0.06 mm in female.

Anterior club-shaped muscular portion of oesophagus 0.46 to 0.5 mm long in male, 0.63 to 0.69 mm in female; 0.09 to 0.1 mm wide in male, 0.12 to 0.15 mm in female. Posterior cylindrical glandular portion of oesophagus 0.4 to 0.44 mm long in male, 0.59 to 0.61 mm in female; 0.09 to 0.1 mm wide in male, 0.13 to 0.15 mm in female.

Nerve ring 0.14 to 0.17 mm from anterior end in male, 0.18 to 0.2 mm in female. Excretory pore 0.36 mm from anterior end of body in male.

Male.—Tail (Fig. 7) 0.14 to 0.15 mm long with bifid tip. Caudal alae long, narrow, beginning at cuticular swelling on ventral side of body and with a maximum width of about 0.03 mm. Six pairs preanal, two pairs small, pedunculated, adanal, and five pairs postanal papillae. Of the latter, the two most posterior pairs are pedunculated and isolated (Fig. 7), while the three anterior pairs are pedunculated and close together in a group. Except for the posterior three pairs of precloacal papillae, which are close together near the cloaca, the precloacal papillae are isolated. Right spicule relatively stout, about 0.4 to 0.46 mm long (Fig. 8) distally, with a lateral barb or prong 0.04 mm long near the tip. Left spicule slender, slightly broader anteriorly, 0.28 to 0.32 mm long.

Female.—Tail (Fig. 9) 0.135 to 0.165 mm long, finger-shaped, with two small cuticular processes at tip. In a specimen 20.2 mm long, the vulva is in front of mid-body, 8.2 mm from anterior end; a distinct lobe-like structure forms lip of vulva.

Camallanus thapari sp. nov. most closely resembles *C. nodulosus* but can be distinguished from it in the number and arrangement of pre- and post-cloacal papillae. In *C. thapari* (Fig. 7) there are six pairs of preanal and five pairs of postcloacal papillae of which three anterior pedunculated postcloacal papillae are close together in a group. The fourth and fifth postcloacal papillae are pedunculated and isolated, while in *C. nodulosus* (Fig. 3) there are seven

pairs of precloacal and six pairs of postcloacal papillae, of which four anterior pedunculated postcloacal papillae are close together in a group, and the sixth is sessile.

C. thapari differs from *C. baylisi* (5) in the possession of five pairs of postcloacal papillae instead of six, and in the possession of a larger left spicule. In *C. thapari* the left spicule measures 0.28 to 0.32 mm whereas in *C. baylisi* it is 0.15 to 0.23 mm.

C. thapari can be distinguished from *C. ranae* (7) in having the most posterior postcloacal papillae pedunculated instead of sessile. *C. thapari* has two cuticular processes (Fig. 9) at the tip of the female tail, which is bifid in *C. ranae*. *C. thapari* differs also from *C. ranae* in the possession of a barb or prong (Fig. 8) 0.04 mm long at the tip of the right spicule.

The new species is named in honor of Dr. G. S. Thapar, Professor of Zoology, Lucknow University, Lucknow, India.

Host.—*Rana tigrina.*

Habitat.—Small intestine.

Locality.—Dacca, East Pakistan.

Key to the Species of the Genus *Camallanus* Railliet and Henry, 1915 from Amphibia

A. Beaded longitudinal ridges in buccal capsule
  (*a*) Six pairs preanal, five pairs postanal papillae..... *C. thapari* sp. nov.
  (*b*) Seven pairs preanal, six pairs postanal papillae..... *C. nodulosus* sp. nov.

B. Nonbeaded longitudinal ridges in the buccal capsule
  I. Vulva anterior to mid-body
    (*a*) Anterior oesophagus larger than posterior oesophagus
      (*x*) One spicule present..... *C. nigrescens* (von Linstow, 1906)
      (*y*) Two spicules present
        i. Six pairs postcloacal papillae, barbs or prongs present at tip of right spicule.... ..... *C. baylisi* (Karve, 1930)
        ii. Five pairs postcloacal papillae, barb or prong absent on tip of right spicule..... ..... *C. ranae* (Khera, 1954)
    (*b*) Anterior oesophagus smaller than posterior oesophagus
      (*x*) Twenty-two to twenty-four longitudinal ridges present in buccal capsule..... ..... *C. multilineatus* (Kung, 1948)
      (*y*) Nine to fourteen longitudinal ridges present in buccal capsule
        i. Three pairs of postcloacal papillae present..... ..... *C. microcephalus* (Dujardin, 1845) R. and H., 1915
        ii. Seven pairs postcloacal papillae, one pair sessile terminal papillae present..... ..... *C. multiruga* (Walton, 1932)
  II. Vulva posterior to mid-body
    (*a*) Larger in size (7.8- to 8-mm male, 10.25-mm female) and having fewer (12 to 15) cuticular ridges in buccal capsule..... *C. pipientis* (Walton, 1935)
    (*b*) Smaller in size and having larger number of cuticular ridges in buccal capsule
      i. Barb or prong at tip of right spicule..... *C. mazabukae* (Kung, 1948)
      ii. Barb or prong absent at tip of spicule..... ..... *C. kaapstadi* (Southwell and Kirshner, 1937)

Procamallanus daccai sp. nov.

(Figs. 10–15)

A large number of male and female specimens of this species were obtained from the small intestine of an unidentified catfish from Dacca, East Pakistan.

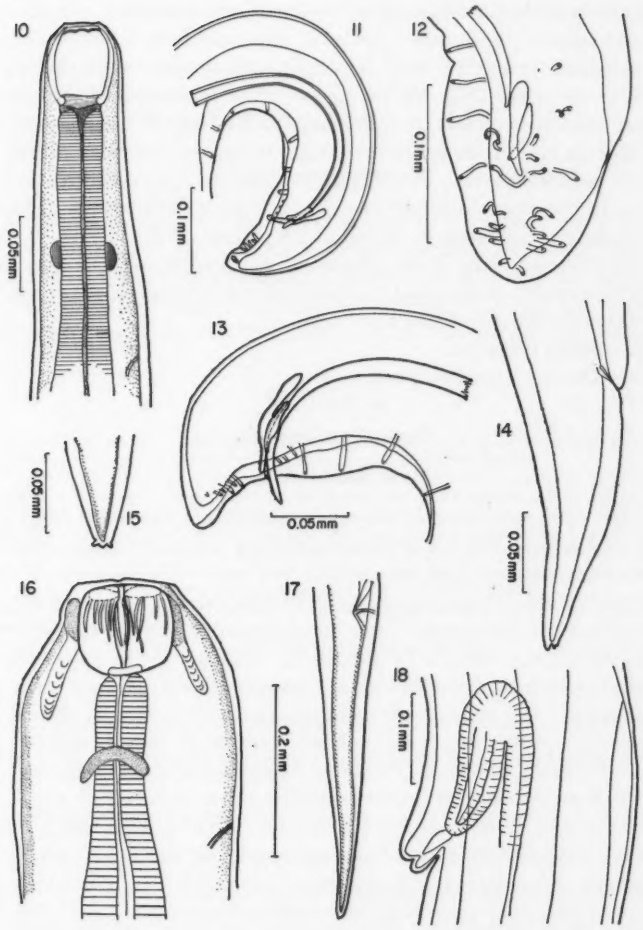
Male 3.55 to 4.5 mm long, female 4.76 to 7.39 mm long. Male 0.1 to 0.125 mm wide, female 0.12 to 0.13 mm wide. Cuticle more striated in female than in male specimens; striations 0.013 to 0.016 mm in male, 0.01 to 0.02 mm apart in female.

Mouth surrounded by two lateral and four submedian papillae. Dorsoventral diameter of male head 0.035 to 0.045 mm, of female 0.04 to 0.048 mm. Brown-yellow buccal capsule with smooth lining, no ridges or leaf crowns, somewhat barrel-shaped, 0.06 to 0.08 mm long in female, 0.05 to 0.06 mm in male; 0.03 to 0.05 mm in width in female, 0.03 to 0.042 mm in male (Fig. 10). Basal ring of buccal apparatus represented by two knob-like structures; lower part of buccal wall thicker than upper anterior part, and posterior margin greatly thickened to form a rim articulating with anterior end of oesophagus. Oesophagus divided into two parts, an anterior muscular club-shaped part, 0.26 to 0.35 mm long and 0.03 to 0.04 mm wide in the female, and 0.325 to 0.625 mm long and 0.042 to 0.05 mm in width in male. Nerve ring 0.12 to 0.13 mm from anterior end of female and excretory pore 0.204 to 0.26 mm situated behind it; the corresponding parts of the male measure 0.125 to 0.13 mm and 0.18 to 0.22 mm respectively, from anterior end.

Male.—Tail (Fig. 11) coiled ventrally and bluntly rounded at tip, 0.05 to 0.07 mm long. Caudal alae well developed. Seven to eight pairs long preanal, one pair adanal, and nine pairs postanal papillae, all pedunculated (Fig. 12). Of the latter, three very small papillae at the sides of the cloacal aperture situated subventrally and one behind the other, and six pairs postanal papillae of which the five anterior postanal papillae are close together in a group, and the sixth most posterior one, very small and isolated. Preanal papillae situated at almost regular intervals and adanal situated laterally at level of cloaca. Right spicule broad at anterior end, narrow and sharply pointed towards posterior end (Fig. 13) 0.36 to 0.48 mm long. Left spicule absent. An accessory piece is present made up of two anterior parts, one slightly broader than the other, and a narrow, pointed, and twisted posterior part, brown-yellow in color like that of the buccal capsule. It is 0.06 to 0.07 mm long.

Female.—Tail (Fig. 14) of female bluntly rounded at apex, terminating in two spinous processes (Fig. 15). It is 0.12 to 0.18 mm long. Vulva situated behind mid-body 2.6 to 3.96 mm from anterior end. Uterus greatly distended with larvae. Some of the eggs seen *in utero* contained a segmenting ovum, others a larva.

Nine species of *Procamallanus* Baylis (2) with a smooth buccal capsule and no transverse or spiral thickenings and no leaf crowns have been reported from fishes and amphibia. Of these, two species are from amphibians, namely *P. slomei* Southwell and Kirschner, 1927 (14) and *P. brevis* Kung, 1948 (10), and the rest from fresh-water and marine fishes, namely *P. laeviconchus* Wedl, 1862 (2), *P. planoratus* Kulkarni, 1935 (9), *P. signai* Yamaguti, 1935 (17), *P. murrayensis* Johnston and Mawson, 1940 (4), *P. lonis* Yamaguti, 1941 (18), *P. annulatus* Yamaguti, 1954 (19), and *P. aspiculus* Khera, 1955 (8).



FIGS. 10–15. *Procamallanus daccai* sp. nov. Fig. 10. Anterior region of male, lateral view. Fig. 11. Male tail, lateral view. Fig. 12. Male tail, ventral view. Fig. 13. Male tail showing tip of longer spicule, lateral view. Fig. 14. Female tail, lateral view. Fig. 15. Female tail, ventral view.

FIGS. 16–18. *Camallanides* sp. Fig. 16. Anterior region of female, lateral view. Fig. 17. Female tail, lateral view. Fig. 18. Vulvar region, lateral view.

Procamallanus daccai sp. nov. (Fig. 11) differs from the above-mentioned species except *P. annulatus* Yamaguti, 1954 (19) in the possession of an accessory piece. It differs from *P. annulatus* in the smaller size of the body, in having the vulva behind the mid-region of the body, in possessing seven to eight preanal, one adanal, and nine postanal, instead of three preanal and five postanal papillae, in having only the right spicule, and in the possession of two cuticular processes (Fig. 15) at the posterior extremity of the tail.

P. daccai resembles closely *P. gubernaculus* Khera, 1955 (8) in having only one right spicule and in the possession of an accessory piece but differs from it and from *P. mehrii* Agarwal, 1930 (1) and *P. bagarii* Karve and Naik, 1951 (6) from India, in the absence of leaf crowns and spiral thickenings in the buccal capsule. It differs also from *P. planoratus* Kukarni, 1935 (9) and *P. aspiculus* Khera, 1955 (8) from India in the possession of an accessory piece, in the number and arrangement of the anal papillae, and in having only a right spicule.

Host.—Catfish (not identified further).

Habitat.—Small intestine.

Locality.—Dacca, East Pakistan.

Camallanides sp.

(Figs. 16–18)

One female specimen from *Hydrophis cyanocinctus* was available for study.

Length 10.4 mm, width 0.4 mm. Cuticle thin, finely striated. Mouth elongated dorsoventrally, with three papillae per side. Buccal capsule (Fig. 16) bivalved, each valve consisting of two chitinoid masses separated by a broad longitudinal groove, thus presenting an appearance of four separate masses. Buccal cavity 0.1 mm long, 0.11 mm wide. Valves bear 12 complete and incomplete longitudinal ridges. Tridents represented by simple pointed chitinous rods of somewhat irregular shape with a dorsal and a ventral chitinoid body lying opposite to edges of buccal valves. Length of rod on right side 0.065 mm, and that on left, 0.09 mm. Posterior ring of apparatus 0.045 mm diameter. Diameter of head, measured dorsoventrally at the corners 0.13 mm.

Oesophagus 0.92 mm long, 0.12 mm wide, divided into two portions, an anterior club-shaped portion 0.42 mm long and 0.06 mm wide and a posterior cylindrical glandular portion 0.5 mm long, 0.12 mm wide. Nerve ring situated 0.19 mm and excretory pore 0.32 mm from anterior end.

Tail (Fig. 17) short, 0.33 mm long. Lips of vulva (Fig. 18) modified into tubular appendage, flattened dorsoventrally, projecting freely from body wall in posterior direction 0.04 mm. This appendage originates a little anterior of mid-body, 4.6 mm from anterior end. Vulvar aperture on ventral side of appendage near its extremity. Vagina runs from a little anterior to opening of appendage.

Baylis and Daubney (3) created the genus *Camallanides* with *C. prashadi* as the type species. Khera (7) divided it into two subgenera, *Camallanides* and *Procamallanides*. *Camallanides* is characterized by the presence of dorsal and ventral chitinoid bodies while *Procamallanides* is based upon their absence.

The present species is close to *C. prashadi* in having dorsal and ventral chitinoid bodies, in the structure carrying the vulva in the female, but differs in being smaller, in the position of the vulva aperture, which is anterior to midbody, and in the position of the vagina which runs a little anterior to the opening of the base of the vulva.

Host.—*Hydrophis cyanocinctus.*

Habitat.—Small intestine.

Locality.—Derma, East Pakistan.

Acknowledgments

This work was carried out during the tenure of a Postdoctorate Fellowship from the National Research Council of Canada at the Institute of Parasitology of McGill University. On behalf of Commander Kuntz I acknowledge the help of Dr. Robert F. Inger, Curator of Reptiles, Chicago Natural History Museum, who provided the identifications of the fishes, amphibians, and reptiles.

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FUNGAL PARASITES OF MOSQUITO LARVAE FROM THE ORIENTAL AND AUSTRALIAN REGIONS, WITH A KEY TO THE GENUS COELOMOMYCES (BLASTOCLADIALES : COELOMOMYCETACEAE)[1]

MARSHALL LAIRD

Abstract

Coelomomyces finlayae n. sp. and *C. macleayae* n. sp. are described from larvae of *Aëdes* (*Finlaya*) *notoscriptus* (Skuse) and *A.* (*Macleaya*) sp. respectively. Both type localities are in Queensland, Australia. *C. stegomyiae* Keilin var. *rotumae* n. var. parasitizes *Aëdes* (*Stegomyia*) sp. on the island of Rotuma, near Fiji. *C. cribrosus* Couch and Dodge and *C. indiana* Iyengar are recorded from new localities, British North Borneo in the first case and Cambodia in the second. New hosts are listed for *C. cribrosus: Culex* (*Lophoceraomyia*) *fraudatrix* Theobald; and *C. stegomyiae: Armigeres obturbans* (Walker). A key to the 21 adequately characterized species of *Coelomomyces* is followed by a brief discussion of the significance of these fungi.

Introduction

Twenty-one species of *Coelomomyces*, and three distinct varieties of one of them, have now been described. There are also a number of records that cannot be carried below the generic level, besides accounts of organisms clearly referable to these fungi although not so recognized by the authors concerned. *C. notonectae* (Bogoyavlensky) parasitizes a Russian hemipteran (1), and an undescribed species has been recorded from *Simulium metallicum* Bellardi in British Honduras (4). With these two exceptions, *Coelomomyces* fungi are known only from mosquito hosts.

Parasitized larvae usually die before or during the fourth instar, through destruction of the imaginal buds, the fat body, and other organs. The genus thus has decided significance for medical entomologists. Nevertheless, the importance of these fungi as factors in the natural regulation of numbers in the Culicidae, has been, like that of other pathogens of these insects, generally disregarded.

Coelomomyces is undoubtedly much more widespread than present data suggest, for records tend to accumulate quickly wherever investigators are alerted to the significance of the genus. Thus 11 (or approximately half) of the known species were described as a consequence of comprehensive mosquito surveys by the State Malaria Control authorities of Georgia, U.S.A., during World War II (2, 3). Several others (including those described for the first time herein) have been discovered in Australasia (where the genus was previously unknown) since 1952, and recent field work there and in Southeast Asia has yielded new host and locality records for known species. The apparent rarity of these fungi in certain areas (including Europe, Asia, South

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America, and much of Africa) and their seeming absence from others (notably subarctic America) reflect a lack of special attention to the genus rather than negative findings.

A recent publication (9) lists hosts and localities for species of *Coelomomyces* named before 1956, and describes two further species. While it is not proposed to repeat these data here, the following supplementary records should be noted: *Aëdes* (*Pseudoskusea*) *australis* (Erichson) (Tasmania) for *C. tasmaniensis* (10), *Aëdes* (*Stegomyia*) *aegypti* L. (Singapore) for *C. stegomyiae*, and *Culex* (*Culex*) *tritaeniorhynchus summorosus* (Singapore) for *C. cribrosus* and *C. quadrangulatus* var. *parva* (11).

However, in view of the current revival of interest in biological control mechanisms in the public health field, a key to the genus supplements the new host and locality records and descriptions of new material presented in this paper.

Methods

Teased-out fragments of parasitized larvae were passed through the alcohol–xylol series, mounted unstained in Stafford Allens' "Sira" medium, and examined by phase contrast. A Zeiss drawing apparatus was used in the preparation of the figures. Fifty consecutive examples of the different stages of each organism were measured.

Systematic Account

Coelomomyces ***finlayae*** n. sp. (Figs. 1–3)

Host: Larval *Aëdes* (*Finlaya*) *notoscriptus* (Skuse).

Habitat: Tree holes.

Locality and date: Springbrook, South Queensland, April 10, 1955.

Two parasitized larvae were received, the entire body cavity from the head capsule to the siphon and anal papillae in the one case exhibiting abundant hyphae but few sporangia, and in the other being crammed with resting sporangia numerous enough to obscure most of the internal organs. Mycelium well developed, the hyphae thin-walled, irregularly branched, and of a thickness of 4.0 to 8.7 μ. Some of the thinner hyphae unbranched, almost straight, and as long as 160 μ, ending in a knobbed swelling from 15 to 20 μ in diameter. Resting sporangia (Figs. 1–3) oval in face view, sometimes rather flattened on one side but usually more or less circular in cross-section. Wall two-layered and of a yellowish-brown color, the inner layer smooth, hyaline, and 0.4 to 0.5 μ thick (av., 0.4 μ) and the outer one minutely pitted, prominently

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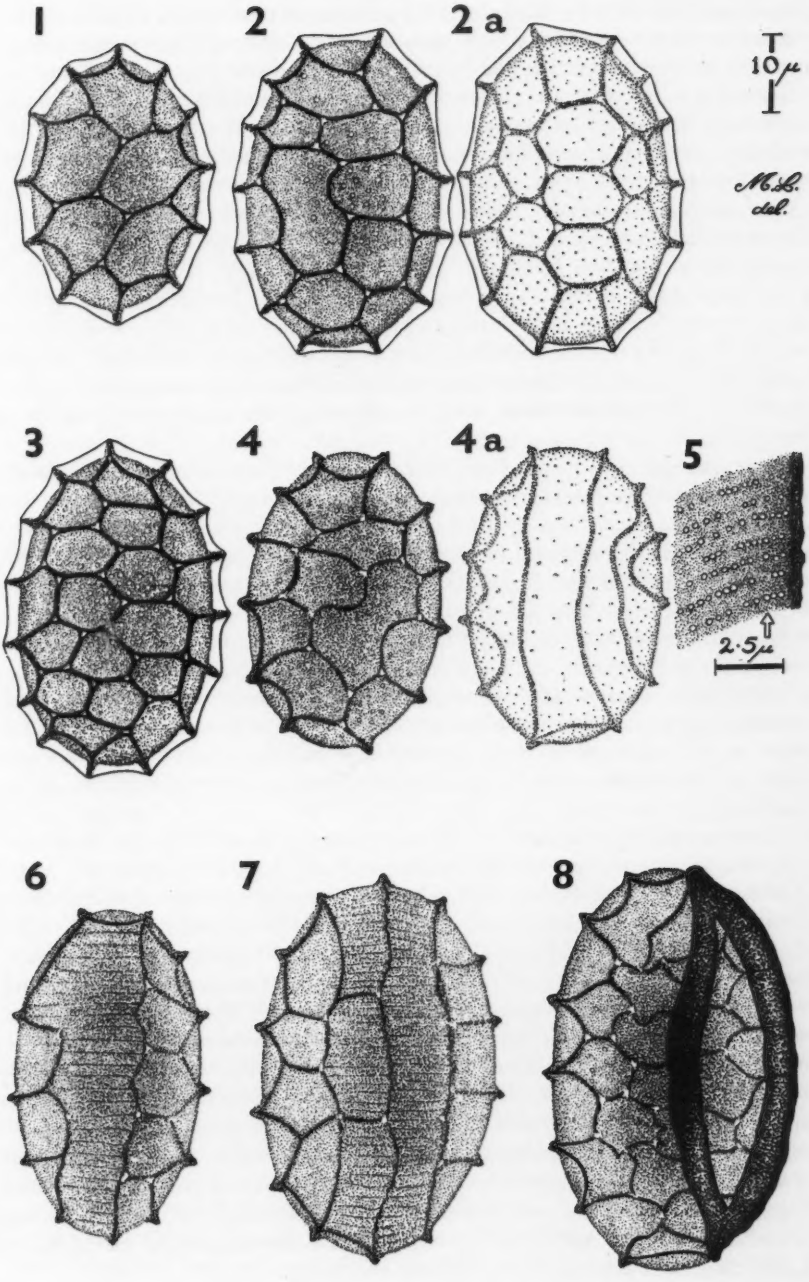
FIGS. 1–8. *Coelomomyces* sporangia. All figures prepared with the aid of a Zeiss drawing apparatus, from mounted but unstained material. All ×1160, except Fig. 5, which is ×4160.

FIGS. 1–3. *C. finlayae* n. sp., Figs. 2 and 2*a* representing opposite faces of the same sporangium.

FIGS. 4–8. *C. macleayae* n. sp., Figs. 4 and 4*a* representing opposite faces of the same sporangium, and Fig. 5 illustrating the rows of pits appearing as transverse striae at lower magnifications (Figs. 6 and 7).


1
2
2a
10μ
M.L. del.
3
4
4a
5
2·5μ
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7
8

ridged, and 0.9 to 1.3 μ thick (av., 1.1 μ) between the ridges. The retained plasma membrane is often conspicuous in optical section (Figs. 1–3), sagging between the ridges of the wall. Sporangia 31.5 to 47.5 μ (av., 37.9 μ) by 17.8 to 27.5 μ (av., 22.4 μ). Narrow, raised ridges 1.4 to 2.9 μ high (av., 2.0 μ), anastomosing to enclose irregular polygonal areas. These are often larger, and thus of course less numerous, on small sporangia (Fig. 1) than on large ones (Fig. 3).

The sporangial ornamentation of this species closely resembles that of *Coelomomyces cairnsensis* Laird (9), other near relatives of which are two incompletely described species, *C. africanus* Walker (15) and *C. ascariformis* van Thiel (14). Resemblance between the species under discussion, and *C. cairnsensis*, extends to the conspicuousness of the old plasma membrane, a feature that was neither mentioned nor illustrated in my description of the latter organism, due to a lack of appreciation of its possible taxonomic value—the presence of the membrane being one of the generic characters of *Coelomomyces* (2).

The parasite of *A. notoscriptus* differs from *C. cairnsensis* in the much smaller size of its resting sporangium (which measures 41.8 to 61.2 μ by 26.5 to 35.5 μ, av. 51.3 μ by 31.7 μ, in the latter species). However, mere size must not be overstressed as a specific character in *Coelomomyces*. Varieties of *C. quadrangulatus* Couch, for example, differ considerably from one another in this respect (3, 11). Of greater significance are certain details of the sporangial ornamentation, notably the more strongly marked ridging of the wall in *C. cairnsensis*, in which the height of the ridges ranges from 3.0 to 5.0 μ. Furthermore, whereas the sporangia of *C. cairnsensis* bear a network of narrow ridges enclosing hexagonal areas on one face and markedly irregular ones on the other, those of the present species do not exhibit marked and constant differences in the ornamentation of their opposed surfaces (Figs. 2, 2*a*).

C. cairnsensis is as yet known only from material from a larva of *Anopheles* (*Myzomyia*) *farauti* Laveran collected near Cairns, North Queensland. The distance of rather less than 500 miles between Springbrook and Cairns is trifling by comparison with that separating locations recorded as positive for *C. stegomyiae* Keilin (Malaya, Rennell in the British Solomon Islands Protectorate, Rotuma), *C. indiana* Iyengar (India, Cambodia, Queensland), and varieties of *C. quadrangulatus* (U.S.A. and Malaya). Neither is a strict host specificity to be assumed, for certain species parasitize representatives of two or more genera of the Culicidae. The type of larval habitat concerned appears to be of more significance. Thus the nine known hosts of *C. indiana* (eight species of *Anopheles* and one of *Aëdomyia*) and the four mosquitoes from which *C. psorophorae* has been recorded (two species of *Psorophora*, one of *Aëdes*, and one of *Culiseta*) all utilize surface waters. Even so, Couch and Dodge (3) saw no incongruity in describing a new variety of *C. quadrangulatus*, a species previously known only from surface-water anopheline larvae, from larval *Stegomyia* originating from small containers.

In the present state of our knowledge, however, it is felt that the differences between the sporangia of *C. cairnsensis* and the *Aëdes notoscriptus* parasite are of sufficient magnitude to warrant the description of the latter as a new species. It is accordingly designated *Coelomomyces finlayae* n. sp., the specific name referring to the subgenus of the host. The type slide and a cotype have been deposited in the collection of the Department of Entomology of the University of Queensland, Brisbane, Australia, and I have retained two cotypes.

Coelomomyces ***macleayae*** n. sp. (Figs. 4–8)

Host: Larval *Aëdes* (*Macleaya*) sp.
Habitat: Tree hole.
Locality and date: Palm Island, North Queensland, April 24, 1955.

This species is described from a single larva exhibiting resting sporangia throughout most of the body, particularly the thorax and abdomen. The larva being a unique specimen required by Dr. Marks for further study, only a small portion of the abdomen was removed and teased out. This contained little mycelium, the few hyphal fragments seen measuring 5 to 9.5 μ in thickness.

Resting sporangia (Figs. 4, 4*a*, 6–8) of oval outline, usually flattened on one side, 34.6 to 53.4 μ (av., 43.2 μ) by 16.8 to 28.1 μ (av., 22.0 μ). Breadth and thickness co-equal. Wall two-layered and of a yellowish-brown color, the inner layer smooth, hyaline, and 0.4 to 0.5 μ thick (av., 0.4 μ), the outer one minutely pitted, ridged, and 1.0 to 1.4 μ thick (av., 1.1 μ). Ridges appearing as equilateral triangles in optical section (Fig. 4, etc.), their height varying from 1.0 to 1.8 μ (av., 1.4 μ); anastomosing to enclose irregular polygonal areas resembling those of *C. finlayae* n. sp. on one side (Figs. 4, 8), the opposite (flattened) side usually having one or more zones bordered by roughly longitudinal ridges (Figs. 4*a*, 6, 7) often extending from end to end of the sporangium. The surface enclosed by these ridges seemingly minutely striated transversely (Figs. 6, 7), although at high magnifications (Fig. 5) it becomes evident that the apparent striae consist of rows of tiny pits. Old plasma membrane not in evidence, but a few of the larger sporangia partly invested by a network of thick strands of a very dark-brown color (Fig. 8).

Sporangia of this species and *C. finlayae* n. sp. resemble one another rather closely. However, those of the *Macleaya* parasite are easily distinguished by the different ornamentation of their two faces. Parallel ridges running the length of the sporangium have not been seen in *C. finlayae* n. sp., and neither have transverse rows of pits like those illustrated in Fig. 5 nor an outermost layer like that shown in Fig. 8. The absence of a conspicuous plasma membrane in the species under discussion further separates it from *C. finlayae* n. sp.

The longitudinal ridges on one face of the present species, together with the appearance of transverse striae between them, recall the ornamentation of *C. uranotaeniae* Couch, a parasite of *Uranotaenia sapphirina* (O.S.) in Georgia, U.S.A. However, both surfaces of the sporangium are similarly ornamented

in *C. uranotaeniae*. *C. lativittatus* Couch and Dodge has longitudinal bands (not ridges) on one side of the sporangium and irregular or transverse bands on the other, and *C. bisymmetricus* Couch and Dodge and (rarely) *C. sculptosporus* Couch and Dodge have sporangial bands so disposed as to give the wall a bilateral symmetry. In the two last-named species, the outline of the sporangium presents a domed, not a spined appearance, due to the thickness of the raised bands.

Differing as it does from other members of its genus, the parasite under consideration is designated *Coelomomyces macleayae* n. sp., the specific name referring to the subgenus of the host. The type slide has been deposited in the collection of the Department of Entomology of the University of Queensland, and I have retained a cotype.

Coelomomyces stegomyiae Keilin var. ***rotumae*** n. var.

Host: Larval *Aëdes* (*Stegomyia*) sp.
Habitat: Discarded cans.
Locality and date: Rotuma Island (north of Fiji), August 6, 1957.

Two of the three larvae on the slide sent to me have the body cavity, from the head capsule to the siphon and anal papillae, packed with boat-shaped resting sporangia. Surface smooth, the brownish-yellow wall pierced by many round pits 1.3–3.3 μ apart. Outer wall layer 1.2 to 1.5 μ (av., 1.3 μ) thick, inner one 0.6 to 0.7 μ (av., 0.7 μ). Over-all measurements, 39.8 to 60.1 μ (av., 50.4 μ) by 19.5 to 30.1 μ (av., 25.5 μ).

Shape, length–breadth ratio of approximately 2:1, the lack of ornamentation and the type of pitting all agree with the characters of *C. stegomyiae* Keilin as previously described (6, 9, 11). This species was already known from the Solomons (9), the record being the most southerly for the genus in the tropical South Pacific until a recent report of a fungal parasite, queried as *Coelomomyces*, from *Culex* (*Culex*) *pipiens fatigans* Wiedemann in New Caledonia (8). The wall is somewhat thinner than usual (1.3 and 0.7 μ as opposed to 1.8 and 0.7 μ), and the sporangium, while conforming to the established size range for this species, is of greater average dimensions than usual: approximately 50 by 25 μ by comparison with 39 to 44 μ by 18 to 24 μ. These facts are held to justify the description of a new variety, *C. stegomyiae* var. *rotumae* n. var., named for the isolated type locality. The type material (the larvae concerned being at the same time paratypes of a new species of the *scutellaris* complex to be described by Dr. J. N. Belkin) is at the School of Public Health and Tropical Medicine, University of Sydney.

Coelomomyces s. stegomyiae Keilin, 1921

Host: Larval *Armigeres* (*Armigeres*) *obturbans* (Walket).
Habitat: Tree hole.
Locality and date: Singapore, July 30, 1958.

Several parasitized *Armigeres* larvae were associated with infected and uninfected larvae of the type host, *Aëdes* (*Stegomyia*) *albopictus* Skuse.

Sporangia from the *Armigeres* larvae have a wall up to 2.6 μ in total thickness having pits 1.5 to 3.3 μ apart, and measure 28.4 to 54.0 μ (av., 41.6 μ) by 16.2 to 29.4 μ (av., 21.7 μ). These figures agree with those already published for examples from Singapore *Stegomyia* larvae (11), and no differences are detectable between hyphae and sporangia from the two hosts collected together on this occasion. Not only the body cavity of these larvae but the alimentary tract as well is packed with sporangia, indicating a particularly heavy concentration of infective material in the habitat. All stages of zoospore formation are evident.

This is the first record of *C. stegomyiae* from a mosquito not belonging to the subgenus *Stegomyia* of *Aëdes*. Affinities between this subgenus and *Armigeres* are well known, for example, their common ability to transmit the virus of dengue.

Coelomomyces indiana Iyengar, 1935

Host: Larval *Anopheles* (*Myzomyia*) *subpictus* Grassi.
Habitat: Irrigation ditch.
Locality and date: Kampot – Phum Swai, Cambodia, June 14, 1958.

The entire haemocoele of the two parasitized larvae from this collection contains large numbers of sporangia. Mycelium is scanty in both cases, the little there is being thin-walled, having short branches, and ranging from 6 to 11 μ in diameter. The younger sporangia are oval and of smooth outline. Some are thin-walled and have vacuolated contents, others, with denser, nucleated contents, having a thicker and refractile wall. These latter measure 38.4 to 47.4 μ (av., 43.5 μ) by 26.1 to 30.4 μ (av., 27.8 μ). Comparable figures from the type material of *C. indiana* are "20×30 – 28×42 μ, sometimes larger" (5). Mature resting sporangia are brownish-yellow, and of circular cross section. They are girdled by very prominent longitudinal bands rising 2.0 to 3.5 μ above the general surface of the wall, the thickness of which ranges from 0.9 to 1.5 μ. The bands often anastomose, and from two to five of them may be seen in face view. They are pierced by great numbers of minute, rounded pits, which give them a striated appearance in side view. The surface of the wall between them is often rasp-like, because of the presence of rows of tiny transverse thickenings. Mature sporangia measure 44.2 to 65.5 μ (av., 57.1 μ) by 30.9 to 41.3 μ (av., 37.4 μ). They are thus rather larger than those described by Iyengar (5), which measured 38 to 60 μ by 25 to 36 μ. In other respects agreement is close, as it is with the smaller mature sporangia of this parasite derived from *Anopheles farauti* Laveran in Queensland (9).

Although the locality is a new one for both genus and species of the fungus, the host is one of those parasitized by *C. indiana* in Bengal (5).

Coelomomyces cribrosus Couch and Dodge, 1947

Host: Larval *Culex* (*Lophoceraomyia*) *fraudatrix* Theobald and *Culex* (*Culex*) *tritaeniorhynchus summorosus* Dyar.

Habitats and dates: Marsh pool (*C. fraudatrix*, July 26, 1956), ponded drain (*C. t. summorosus*, July 26, 1956), small pond (*C. t. summorosus*, August 2, 1956).

Locality: Jesselton, British North Borneo.

Three larvae of *C. t. summorosus* and two of *C. fraudatrix* were heavily parasitized by this fungus, masses of resting sporangia packing the entire body cavity. Mycelium abundant, the hyphae 5 to 15.2 μ thick. Sporangia oval to allantoid, circular in cross-section, and measuring 46.2 to 68.9 μ (av., 61.2 μ) by 27.1 to 44.1 μ (av., 37.8 μ) in the case of *C. t. summorosus*, and 53.5 to 68.0 μ (av., 62.3 μ) by 31.5 to 39.1 μ (av., 36.9 μ) in that of *C. fraudatrix*. Wall yellowish, two-layered, the outer layer 2.0 to 3.8 μ (av., 3.1 μ) and the inner one 0.4 to 0.7 μ (av., 0.6 μ) in thickness. Both sides of the sporangium with from 7 to 25 circular or oval thin areas pierced by pits 0.3 to 0.65 μ in diameter, each thin area surrounded by a broad, low mound.

Sporangia of the type strain of *C. cribrosus* measure 42 to 71 μ by 24 to 42 μ (3). Singapore examples measure 47.2 to 66.8 μ (av., 57.0 μ) by 31.5 to 43.0 μ (av., 37.2 μ) (11). Details of ornamentation correspond in all cases, and the rather greater breadth range of the hyphae in Bornean material (5 to 15.2 μ, as compared with the 6 to 11 μ of the type) is not of taxonomic significance.

The *Coelomomyces* from Jesselton is thus identified as *C. cribrosus*, both previous records of which concern mosquito larvae from surface waters, two species of *Anopheles* in Georgia, U.S.A. (3), and *C. t. summorosus* at Singapore (11).

Key to the Species of *Coelomomyces*

(The characters are those of the sporangium except where otherwise noted)

1. Surface unornamented 2
   Surface ornamented 8
2. Wall unpitted 3
   Wall pitted 5
3. Wall proper single-layered (?) ..... *C. notonectae* (Bogoyavlensky, 1922)
   Wall proper double-layered 4
4. Measurements at least 50 by 30 μ ..... *C. walkeri* van Thiel, 1954
   Measurements never attaining 50 by 30 μ ..... *C. solomonis* Laird, 1956
5. Measurements often exceeding 90 by 60 μ. Pits often anastomosing 6
   Measurements never attaining 90 by 60 μ. Pits always single 7
6. One side often slightly flattened. Hyphae 7.5 to 10 μ thick .. *C. psorophorae* Couch, 1945
   One side always completely flattened or concave. Hyphae 3.0 to 6.0 μ thick ..... *C. tasmaniensis* Laird, 1956
7. Pits circular in surface view and on focussing downwards ..... *C. stegomyiae* Keilin, 1921*
   Pits slit-like in surface view, star-like on focussing downwards ..... *C. keilini* Couch and Dodge, 1947
8. Ornamentation of round to elongate pits only ..... *C. punctatus* Couch and Dodge, 1947
   Ornamentation otherwise 9
9. Ornamentation of round to elongate pits and raised bands separated by grooves ..... *C. dodgei* Couch, 1945
   Ornamentation otherwise 10

*With var. *rotumae* n.var., characterized by its larger than normal sporangia.

10. Ornamentation of wide, raised bands, dome-like in optical section 11
    Ornamentation of narrow, raised ridges, spine-like in optical section 16
11. Circular in face view, bands concentric or characteristically so ..... *C. anophelesica* Iyengar, 1935
    Oval in face view 12
12. Ornamentation of the two faces always dissimilar, one face bearing longitudinal bands and the other transverse or irregular ones ... *C. lativittatus* Couch and Dodge, 1957
    Ornamentation of both faces similar, or usually so 13
13. Bands longitudinally disposed, anastomosing ..... *C. indiana* Iyengar, 1935
    Bands otherwise disposed 14
14. Bands forming an irregular network 15
    Bands alternately high and low, one side of the sporangium appearing smooth in face view, the other exhibiting alternating high and low domes (the raised bands seen in optical section) on either side of a central mammate structure ..... *C. bisymmetricus* Couch and Dodge, 1947
15. Network of raised bands enclosing a few oblong or elongate (rarely circular) areas ..... *C. sculptosporosus* Couch and Dodge, 1947
    Network of raised bands enclosing 3 to 20 or more circular to oblong areas ..... *C. cribrosus* Couch and Dodge, 1947
16. Ridges longitudinal 17
    Ridges forming an irregular network 20
17. Pits in transverse rows 18
    Pits not so disposed 19
18. Usually four longitudinal ridges, quadrangular appearance in end view ..... *C. quadrangulatus* Couch, 1945*
    Seven or eight anastomosing ridges ..... *C. uranotaeniae* Couch, 1945
19. Five or six longitudinal ridges, pentangular appearance in end view ..... *C. pentangulatus* Couch, 1945
20. Faces usually differently ornamented 21
    Ornamentation of both faces similar 22
21. The ridges of one face enclosing hexagonal to irregular areas, the innermost ones of the other often strongly convoluted ..... *C. cairnsensis* Laird, 1956
    The ridges of one face enclosing irregular polygonal areas, some of those of the other longitudinal ..... *C. macleayae* n. sp.
22. Ridges of both faces enclosing irregular polygonal areas ..... *C. finlayae* n. sp.

Discussion

The above key disregards two inadequately known species, *C. africanus* Walker and *C. ascariformis* van Thiel. The sporangia of *C. africanus* measure 25 to 35 μ in length and appear wrinkled, but it is not clear from the description (15) whether this is due to ornamentation or to the crinkling of a smooth-walled sporangium by reagents. Van Thiel (14) proposed the name of *C. ascariformis* for an unnamed *Coelomomyces* recorded by Manalang (12) in the Philippines and by Walker (15) in Africa. However, it is likely that Manalang's parasite is referable to *C. indiana* Iyengar, and *C. ascariformis* is therefore understood as designating the species having sporangia ranging from 45 to 60 μ in length and bearing raised bands, allocated by Walker to his type 4 (9). Muspratt (13) illustrated what he considered to be a similar organism (his type *b*) from anophelines including Walker's host, *Anopheles* (*Myzomyia*) *gambiae* Giles. From the appearance of the ornamentation as shown in Muspratt's Plate I, Fig. 2, and the length of the sporangium (50 to 70 μ), there is a possibility that this fungus is closely allied to *C. cribrosus*.

*With these varieties: var. *irregularis* Couch and Dodge, 1947 (characterized by a crenate and very irregular outer sporangial wall); and var. *lamborni* Couch and Dodge, 1947, and var. *parvus* Laird, 1959 (respectively having larger and smaller sporangia than the type).

The key clearly reveals some of the shortcomings in our knowledge of the genus *Coelomomyces*. First of all, although the form and size of the hyphae have taxonomic value, the mycelium of many of the species remains undescribed. Then, the dichotomy under "wall unpitted" for those species having a smooth, unornamented sporangium, must be based on whether the wall is single or double-layered—Bogoyavlensky (1) having figured the sporangial wall of (*Zografia*) = *Coelomomyces notonectae* as single-layered. A two-layered wall is regarded as one of the generic characters of *Coelomomyces* Keilin as emended by Couch (2). Nevertheless, although it seems highly probable that Bogoyavlensky overlooked a thin inner wall (7), the other possibility must be conceded until such time as *C. notonectae* has been rediscovered and redescribed. Should the wall prove to be two-layered there is little to choose between this species and *C. solomonis*, which, in its turn, is separated from *C. walkeri* by size alone. Further studies might well disclose synonymy here. Again, in certain instances two different species of *Coelomomyces* have been described from a single host species, or even from the same individual mosquito (*C. punctatus* and *C. quadrangulatus*, *C. keilini* and *C. dodgei*, *C. quadrangulatus* var. *lamborni* and *C. stegomyiae*). This surely invokes a considerable measure of coincidence.

Increasing interest is being taken in the possible role of *Coelomomyces* in the biological control of mosquitoes. Although as already indicated, knowledge of the geographical distribution of the genus is far from complete, enough data have accumulated to reveal that these fungi are of unusually high incidence in some areas, while altogether absent from others. Thus Couch and Dodge (3) recorded *C. dodgei* from 58 locations in Georgia, U.S.A., and from personal observations and current studies by Dr. D. H. Colless it is evident that *C. stegomyiae* is established in from 3 to 5% of larval habitats of *Aëdes albopictus* on Singapore Island. Research in Rhodesia during 1941–45 revealed that throughout this period *Coelomomyces* (particularly a species resembling *C. indiana*) reduced an *Anopheles gambiae* population by approximately 95% (13). On the other hand, there are localities, including isolated Pacific atolls, where intensive sampling has failed to reveal any infections. The rapidly increasing magnitude and complexity of insecticide resistance problems is forcing the investigation of alternative and auxiliary control procedures. Towards the end of 1958 a field experiment employing Singapore-derived *C. stegomyiae* in the biological control of *Aëdes* (*Stegomyia*) *polynesiensis* Marks was begun in the Tokelau Islands, South Pacific, under the auspices of the World Health Organization and with the co-operation of the New Zealand Government (16).

Much of the work on *Coelomomyces* has been accomplished by zoologists, intrigued by parasites so lethal to a family of insects of first importance in the field of public health. Systematic and biological problems now confronting us—notably those of transmission and host specificity, bearing as they do upon the significance of these organisms in the natural regulation of mosquito populations—merit early and detailed attention on the part of qualified mycologists. In the meantime, though, studies of the genus should

not be suspended. It is submitted that, pending systematic botanical investigations, least confusion will be caused if sporangial morphology and size continue to be regarded as the chief specific criteria for these fungi.

Acknowledgments

Australian specimens (those from Palm Island, North Queensland, being collected by Dr. M. Josephine Mackerras) were sent to me by Dr. Elizabeth N. Marks of the University of Queensland. My thanks are due to her for this kindness, and for sending me additional material obtained by Mr. S. Nelson at Rotuma. I am also indebted to Dr. D. H. Colless of the University of Malaya, who provided all the parasitized larvae from the Oriental Region.

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ANOMALIES OF ONCOTAXY IN TWO SPECIES OF ECHINOCOCCUS FROM NORTH AMERICA[1]

G. LUBINSKY

Abstract

Anomalies of oncotaxy of adult and larval scoleces of *Echinococcus multilocularis* and *E.* aff. *granulosus* from North America are described. It is pointed out that some of these anomalies affect characters usually believed to be characteristic of species. Abnormal oncospheres of *E. multilocularis* with 2, 7, and 12 hooks are described and the probable origin of the giant oncospheres is discussed.

The oncotaxy and the size and shape of rostellar hooks are widely used as taxonomic characters of specific value and, to some extent, as characteristics of higher taxa. They are of considerable importance in differentiating species of the genus *Echinococcus*. The evaluation of the taxonomic significance of the oncotaxy and of the morphological characters of individual hooks in this genus necessitates the study of their anomalies.

The present paper is based on the examination of larval scoleces of *Echinococcus multilocularis* Leuckart (Rausch strain) from experimentally infected muskrats and cotton rats, and of adult scoleces of the same species from dogs fed hydatid cysts from muskrats. Also examined were larval scoleces of *Echinococcus* aff. *granulosus* Batsch from Canadian moose and reindeer, as well as adult scoleces of the same species from dogs fed hydatid cysts from reindeer and moose. A few cysts of human origin were examined. Scoleces were cleared in lactic acid, which dissolves the calcareous corpuscles, and examined in temporary mounts. Drawings were made with the aid of a Zeiss drawing prism.

The examination of the oncotaxy of several hundred larval and adult scoleces has shown that all the populations examined contained scoleces with an uneven number of hooks, a phenomenon described by Vogel (21) in *E. multilocularis*. The proportion of crowns with uneven numbers of hooks usually varied between 20 and 30%. The most common cause of the unevenness of this number was the absence of one or several small hooks. Figure 1 represents a part of the crown of 35 hooks of an adult *Echinococcus* bred from a hydatid cyst of a reindeer. In the place where the handle of the absent hook should have been, there is a group of spherules 1 to 3 μ in diameter. Near the apex of the rostellum there is an irregular mass about 12 μ in diameter with the optical properties of the substance of the hooks.

The replacement of the manubrium of a missing hook by a group of globules is seldom observed. Usually the missing hooks are represented by rudiments of their guards (Figs. 5, 6, 8) or both guards and handles (Fig. 4). These rudiments vary from small oblate spheroids (Fig. 5) to hook-like structures

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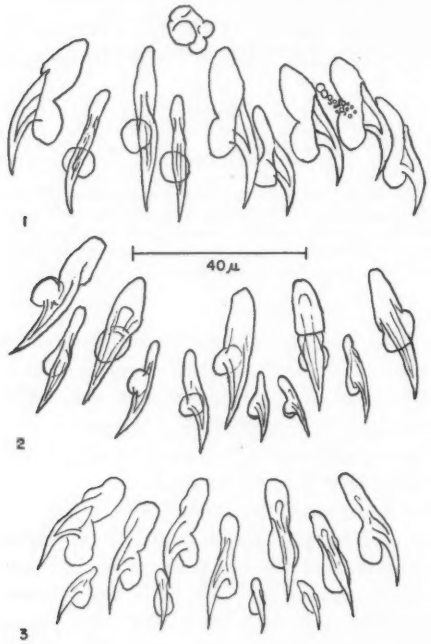
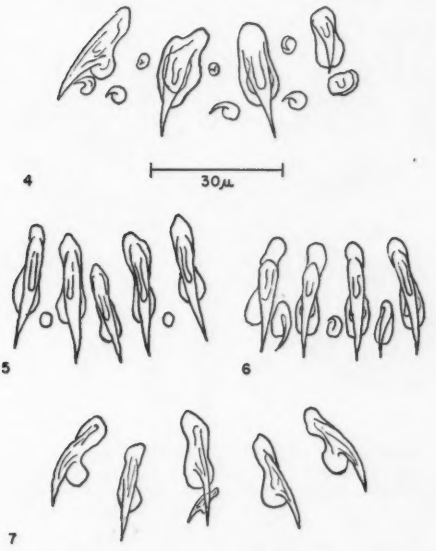


FIGS. 1–3. Anomalies of oncotaxy in adult cestodes of the reindeer strain of *Echinococcus*. Fig. 1. Deletion of a small hook. Fig. 2. Duplication of two small hooks. Fig. 3. Incomplete third row of hooks.

(Figs. 4, 6, 8). A much more rare occurrence is the disintegration of the rudiments of small hooks into an irregular group of minute hook-like structures and granules (Fig. 9). On several occasions I have observed a complete disappearance of the small hooks (Fig. 8 upper part, Fig. 11). The complete or partial deletion of the small hooks is usually accompanied by an increase in size of the large hooks (Fig. 11). The crown represented in this last figure consisted of only 10 hooks with heavy nodular bases, resembling those of the hooks of adult *Echinococcus*.

Another anomaly of oncotaxy is the complete replacement of both small and large hooks by numerous small hooklets and granules of irregular shape. The hooklets are not differentiated into a handle and guard, and are not oriented as to the longitudinal axis of the scolex. Figures 12 and 13 represent parts of such larval crowns of *E. multilocularis*, in which, however, a few large hooks are still present. Such anomalies are much more frequent in larval scoleces than in those of adult cestodes, probably because they interfere with the fixation to the wall of the gut and are thus of negative survival value for the adult cestodes.

The anomalies of oncotaxy resulting in the increase of the number of hooks are less frequent than those leading to the decrease in this number. One of the

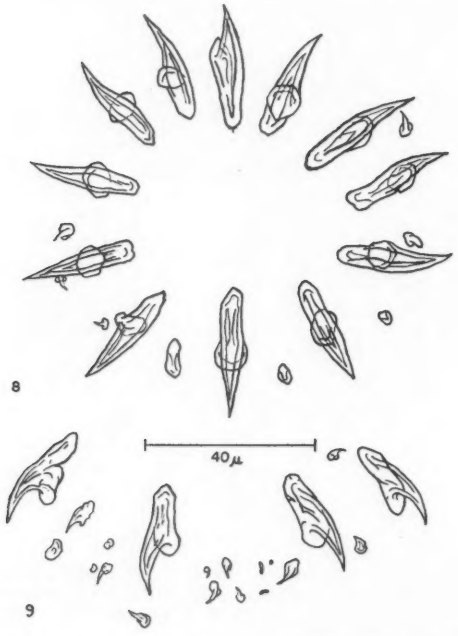


FIGS 4–7. Anomalies of oncotaxy in the adult *E. multilocularis* (Rausch strain). Fig. 4. Incomplete absence of small hooks with persistence of rudiments of handles and guards. Figs. 5–6. Deletion with persistence of guards only. Fig. 7. One accessory hook near the handle of a large hook.

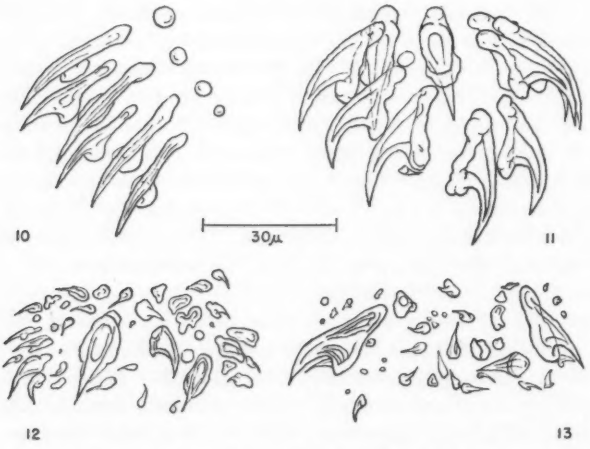
most common of such anomalies is the appearance of small supernumerary hooklets distally to the guards of the large hooks (Fig. 7, Fig. 8 left side). Such hooklets are never numerous and accompany only a small proportion of the large hooks. The presence of accessory hooks is sometimes combined with the absence of small hooks and with other anomalies of oncotaxy. Another rare anomaly leading to the increase of the number of hooks is the duplication of small hooks (Fig. 2). This anomaly usually affects only a few small hooks of the crown. I have not seen crowns with duplication of all the small hooks. Almost as rare as the duplication is the appearance of a third row of hooks distally to the small hooks (Fig. 3). The hooks of the third row form oblique rows with the small and large hooks. The maximum number of hooks of the third row, 12, was seen in a scolex with 18 large and 18 small hooks, the total number of hooks being thus 48. Two other scoleces also with an incomplete third row of hooks had 45 and 46 hooks respectively.

I have not seen accessory rows of hooks situated anteriorly to the large hooks but on several occasions have seen sphaeroids situated anteriorly to the handles of both the large and small hooks (Fig. 10). It is hard to decide whether these represent rudiments of a supernumerary row of hooks.

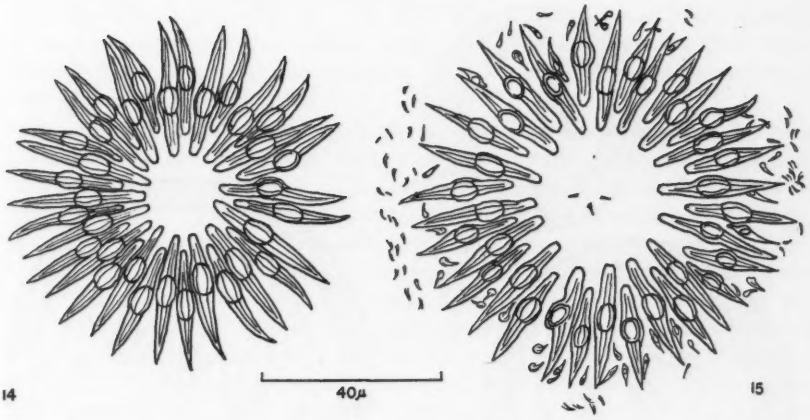
Abnormal scoleces may sometimes differ from normal ones in characters usually evaluated as specific. Figures 14 and 15 represent two crowns of hooks from a hydatid cyst from a human lung. The first is composed of 32 hooks (16 large and 16 small) and is typical of *E. granulosus*. The second crown



FIGS. 8–9. Anomalies of oncotaxy in larval *E. multilocularis* from a muskrat. Fig. 8. A crown of 12 large hooks with complete absence of small hooks in its upper part and incomplete absence in its lower part. Fig. 9. Replacement of small hooks by irregular groups of hooklets.



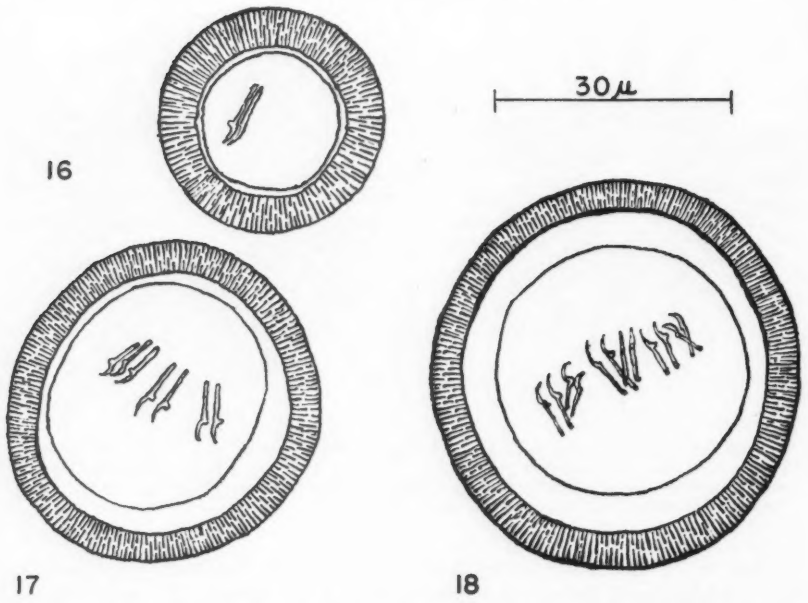
FIGS. 10–13. Anomalies of oncotaxy in adult (Fig. 10) and larval (Figs. 11–13) *E. multilocularis*. Fig. 10. Spherules proximally to the handles of hooks. Fig. 11. A crown of 10 large hooks. Figs. 12–13. Irregular oncotaxy with persistence of a few large hooks.



FIGS. 14–15. Two crowns of larval scoleces from a human cyst. Fig. 14. Normal crown of 32 hooks typical of *E. granulosus*. Fig. 15. A crown of 34 large and small hooks with numerous accessory hooklets resembling that of *E. lycaontis* Ortlepp, 1934. Note the small hooklets near the center of the crown.

consists of 16 large and 18 small hooks (2 small hooks being doubled), and has two additional belts of irregularly arranged hooklets. The first belt consists of hooklets 7 to 10 μ long, with dilate bases, not oriented in relation to the main axis of the scolex. The second incomplete belt consists of irregularly arranged slightly curved hooklets of almost uniform width, 3 to 5 μ long. The second scolex thus has an oncotaxy closely resembling that of *E. lycaontis* Ortlepp, 1934 (17). Peculiar to the crown represented in Fig. 15 is the presence near its center of small thorn-like hooklets. Such hooklets are never numerous; they occur in a very small percentage of scoleces.

In examining scoleces a few oncospheres of *E. multilocularis* with abnormal numbers of hooks were incidentally found. Their number was low, only five such oncospheres being found in an examination of over 60 mature specimens of *E. multilocularis* containing about 10,000 ova—a much lower frequency than that found in the reindeer strain of *Echinococcus* (9). Of these oncospheres one was 2-hooked, one 7-hooked, and three 12-hooked (Figs. 16–18). The volume of the last was about twice that of the normal hexacanths. In the previous paper (9) the occurrence of giant 12-hooked oncospheres was tentatively interpreted as the result of fusion of two developing eggs in the ootype. A perusal of the literature has shown that the fusion of eggs or of early embryos is known to occur in several phyla. Fusion of embryos up to the planula stage in the hydromedusa *Mitrocoma annae* with subsequent development of hydrorhyza was reported by Mechnikov (12) in 1886. In 1892 Herbst (6) observed the fusion of plutei of *Sphaerechinus*. A few years later Morgan (13) described the fusion of developing ova (up to the blastula stage) of *Sphaerechinus*, and was able to influence the frequency of this phenomenon, but not to bring it under complete control. The developing gastrulae and plutei were abnormal



FIGS. 16–18. Anomalous oncospheres of *E. multilocularis*. Fig. 16. A 2-hooked embryo. Fig. 17. A 7-hooked embryo. Fig. 18. A 12-hooked giant oncosphere.

and showed mostly duplications of structures. The development of normal plutei from fused developing ova, up to the blastula stage, was first described by Driesch (3) in 1900, and his subsequent experiments on fusion, in 1910 (4). Heteroplastic fusion of echinoid embryos was achieved by Bierens de Haan (1) in 1913. Nusbaum and Oxner (14) observed spontaneous fusion of eggs of *Lineus ruber* (Nemertea) and later (15) described their further development.

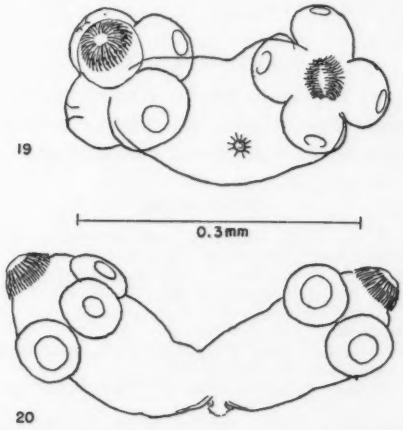
Mangold (10) was the first to fuse embryos of vertebrates experimentally. Working with *Triton taeniatus* he produced giant embryos, both normal and abnormal, in apposing ova at the stage of two blastomeres. Heteroplastic fusion of early embryos of *T. taeniatus* and *T. alpestris* was reported by Mangold and Seidel (11) in 1927.

Although in these experiments the apposed ova formed a single embryo, the cytoplasms of the components did not fuse. A genuine fusion of two or several fertilized eggs of *Ascaris megalocephala* was described by zur Strassen (19) in 1898. Spontaneously, and under the influence of low temperatures, ova of this nematode fused and formed simplastic masses with several nuclei. Multicentric mitoses led to random distribution of chromosomes and subsequent degeneration. However, some products of fusion of two ova developed normally, producing giant tetraploid embryos. Giant eggs were recorded in turbellarians by Sekera (18) and in cestodes by Venard (20) (in *Dipylidium caninum*), by Ionina (7) (in *Hymenolepis nana*), and by Yoshino (22, 23) (in *Taenia solium*). The cytology and structure of the giant embryos of cestodes

was, however, not examined. The fact that the embryonic hooks develop intracellularly, each being formed by one cell (16), and that the giant oncospheres have 12 hooks, may indicate that in such embryos the number of cells is doubled.

Doubling of structures as the result of fusion of ova was observed by Nusbaum and Oxner (14) in the nemertean *Lineus ruber* and produced experimentally by Bierans de Haan (2) in echinids and by Mangold (10) and Mangold and Seidel (11) in amphibians. It is therefore justifiable to pose the question "to what extent the doubling of structures, not infrequently observed in both larval and adult cestodes, may be brought in connection with the giant ova?" This question, which has never been studied experimentally, is not easy to answer, because doubling of structures may be, and mostly is, caused by factors other than fusion of ova. However, the frequency of the occurrence of cysticerci of *Taenia saginata* with two scoleces (8) is compatible with the assumption of their origin from giant oncospheres.

Doubling of larval scoleces of *Echinococcus* was described by Gaschen (5). A colleague, Dr. G. Webster, has given me scoleces of *Echinococcus* joined in pairs, which she found in a human cyst from Canada. Such scoleces (Figs. 19, 20) comprised less than 1% of the total number of holdfasts found in the cyst. This indicates that the causes of duplication of these scoleces are local and not proper to the entire cyst. The fusion of ova may be expected to result in duplication of the number of scoleces—a phenomenon which in *Echinococcus* cannot be observed because of the polyembryony peculiar to this genus. The causal analysis of duplication of structures in cestodes, requiring the application of both cytological and experimental methods, is a promising field for research. Some peculiarities of cestodes, e.g., the sex-linked duplication of structures in *Dioecocestus*, may also be of considerable genetic interest.



FIGS. 19–20. Scoleces of *Echinococcus* joined by their bases (from a human cyst).

Discussion

The anomalies of oncotaxy of the scoleces described in this paper can be classified as follows:

1. Anomalies increasing the number of hooks
   - (*a*) Accessory hooks near the guard of the large hooks
   - (*b*) Accessory (third) row of hooks
   - (*c*) Duplication of small hooks
   - (*d*) Irregularly distributed minute hooks at the periphery of the crown
   - (*e*) Minute hooks near the center of the crown
2. Anomalies decreasing the number of hooks
   - (*a*) Partial deletion of small hooks
     - (*i*) With rudiments of handle and guard remaining
     - (*ii*) With rudiments of guard only remaining
     - (*iii*) With replacement of small hooks by a group of hooklets and irregular grains
   - (*b*) Complete deletion of small hooks
3. Transformation of the crown into a belt of irregular hooklets and spherules
   - (*a*) With a few large hooks remaining
   - (*b*) With complete absence of normal hooks

These anomalies greatly increase the range of variability of the number of hooks in the species of *Echinococcus* examined. In crowns with a third row of hooks this number may reach 48, whereas in those with only one row of hooks, 10. The anomalies of the first group, those increasing the number of hooks, were more common in strains from Canadian Cervoidea, whereas those reducing their numbers were usual in the Rausch strain of *Echinococcus multilocularis*. Whether this difference is peculiar to the strains examined or characteristic of the species to which these strains belong cannot be decided on the basis of the material at hand.

Acknowledgments

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STUDIES IN THE MODE OF ACTION OF ROYAL JELLY IN HONEYBEE DEVELOPMENT

II. RESPIRATION OF NEWLY EMERGED LARVAE ON VARIOUS SUBSTRATES[1]

R. W. SHUEL[2] AND S. E. DIXON[3]

Abstract

Measurements were made of oxygen uptake and carbon dioxide evolution, during the first 24 hours of life, by larvae on substrates of different age and type. These substrates included royal jelly, the pharyngeal secretion fed to larvae destined to become queens, which varied in age from 0 to 96 hours; the pharyngeal secretion fed to young larvae destined to be workers, which varied in age from 0 to 60 hours and which was termed "worker jelly"; and the food supplied to older worker larvae, composed of a pharyngeal secretion modified by an admixture of honey and pollen, and termed "modified jelly". The pattern and magnitude of oxygen uptake was similar on all substrates. Net carbon dioxide evolution by larvae on royal jelly or modified jelly was highly positive; net carbon dioxide evolution by larvae on worker jelly was slightly negative. Microchemical analyses showed that royal jelly differed in composition from worker jelly and modified jelly. The composition of royal jelly remained relatively constant with age. The addition of sugars to worker jelly produced an increase in carbon dioxide output which was nullified by the further addition of an extract of the water-soluble acids of royal jelly. The differences observed in carbon dioxide evolution by young larvae on worker jelly and royal jelly are considered to be an expression of the initiation of female dimorphism. An hypothesis of nutritional balance is advanced to account for this dimorphism.

Introduction

Sexual dimorphism in the honeybee, which is a function of nutrition, seems to be established within the first 3 days of larval life (13).

Melampy and Willis (9), using the direct Warburg method for oxygen, found that consumption by 2- to 3-day old honeybee larvae which were being reared to queens was 50% greater than in larvae destined to be workers. Consequently in the present work, respiration has been used as the early, measurable criterion of this dimorphism. Respiration has been measured on larvae developing on various substrates, including both the naturally occurring diets of the larvae and artificially modified diets.

The Cartesian diver apparatus, capable of measuring in the range of a fraction of a microliter of gas exchange, has obvious advantages over other manometric methods. Its use in the present experiments has permitted measurement of the respiration, during the first 24 hours, of single larvae.

Materials and Methods

The apparatus used was described in the first paper of this series (3). All experiments were conducted at a temperature of 35° C, simulating that in the brood area of a developing colony.

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[2]Department of Apiculture.
[3]Department of Entomology and Zoology.

Collection of Food

Secretions of the pharyngeal glands of worker bees form the basis of the diet of honeybee larvae. Larvae destined to become queens are fed only with pharyngeal secretion, which in accordance with common usage will be referred to as royal jelly. The pharyngeal secretion given to worker larvae will be referred to as "worker jelly". After the third day of larval life nectar and pollen are added to the diet (13); this worker diet we refer to as "modified jelly". The general term "worker diet" will be used to denote both "worker jelly" and "modified jelly".

Royal jelly was collected by the method of Smith (16). Artificial queen cells were grafted into a colony and royal jelly of a known age was subsequently removed by aspiration. Worker diet was collected in a similar manner from brood combs which contained larvae of a known age.

Royal jelly and worker diet were further classified into three groups corresponding to the age of the secretion: 0–30 hours, 40–60 hours, and 72–96 hours.

Freshly gathered substrate takes up oxygen. Consequently material stored at −17° C for several months was used for all experiments.

Manipulation of Larvae

The study was initiated during the winter months. Larvae were obtained from a small colony maintained in a room where the temperature was controlled at 26 plus or minus 3° C. After May 1 the experimental material was obtained from colonies kept out-of-doors.

Larvae were obtained from combs containing a mixture of eggs and young brood. Eggs about to hatch show movement and have a humped dorsal surface. By observing such eggs larvae can be obtained directly upon emergence. The bottom of a cell with its attached larva was removed with a cork borer, and the larva was then transferred to a fine glass rod. This rod was inserted into the charging apparatus previously described (3) and the larva deposited in the bottom of the diver containing the substrate. This method of manipulating larvae resulted in very few casualties.

Respiratory Measurements

Oxygen consumption was determined directly using 0.5 *N* sodium hydroxide seals for the absorption of carbon dioxide.

Data on carbon dioxide production were obtained by using 0.5 *N* sulphuric acid seals. Because parallel measurements of carbon dioxide and oxygen on two substrates necessary for accurate estimates of respiratory quotients are not practical with this apparatus, oxygen values were not used to estimate carbon dioxide evolution. The change in the volume of gas in the system in the presence of sulphuric acid is therefore reported as "net carbon dioxide evolution" (Tables II, IV). This method of reporting should be valid, as carbon dioxide retention by larvae is negligible (9). Nor should the substrate, which has a pH of about 4.0 (3), retain carbon dioxide.

Experimental Design

In order that samples might be representative, each type and age of substrate was collected on at least two and in some cases three occasions. Daily samples in turn represented at least two combs. Each comb represented many hundreds of individual secretions.

A simple analysis of variance design was used for the oxygen determinations. Six determinations were made for each type and age of substrate. Worker diet and royal jelly of similar age were compared on each day.

Group comparisons with the '*t*' test were used for the treatment of carbon dioxide data.

As the Cartesian diver apparatus originally accommodated only six, and later eight, divers the number of daily comparisons was limited. Two types or ages of substrates were compared on each day. The groups under comparison were varied from day to day over the experimental period.

Chemical Methods

The small amounts of larval food available necessitated the use of micro-analytical methods. Protein and sugar determinations were made on wet samples and converted to a dry weight basis using refractive indices as the measure of total solids. Refractive indices were in excellent agreement with dry weights determined directly on oven-dried or lyophilized material. Lipid analyses were made on samples which were lyophilized, then kept for 48 hours *in vacuo* over phosphorus pentoxide at 35° C.

Included in the analyses were the samples used in the respiratory measurements.

A micro-Kjeldahl method was used for nitrogen determination (2). Nitrogen content was converted to % protein by multiplying by 6.25. The method of Somogyi (18) for glucose was used for determining reducing substances, of which about 94% are sugars (19). There is virtually no sucrose in larval food (author's unpublished data).

Lipid determinations were made in three ways: (*a*) lyophilized material was Soxhlet-extracted with dry, peroxide-free ether for 24 hours and the loss in weight determined; (*b*) the ether extract from (*a*) was evaporated to dryness, and taken up in toluene. Aliquots, after evaporation, were dissolved in 100 μl of ethanol and titrated in a CO_2-free atmosphere against 0.02 *N* tetramethyl ammonium hydroxide, with thymol blue as the indicator; (*c*) other aliquots of the toluene solution used in (*b*) were saponified by the ultra-micro method of Schmidt-Nielsen (4) and titrated.

Because water-soluble acids are known to constitute a large portion of the water-soluble fraction of royal jelly (19), the residue from the ether extraction (*a*) was also titrated, after suspension in water.

In addition to the titrations on lyophilized and fractionated material, untreated jelly was suspended in water and titrated. Solution of the untreated jelly was complete by the end of the titration.

Both ether-soluble and water-soluble acid fractions were used to alter diets artificially. The ether extract was obtained from lyophilized royal jelly

by the procedure described above. For the water-soluble acid fraction, an extract of royal jelly from which the ether-soluble fraction and the protein had been removed (1) was passed through an anion-exchanger column (Amberlite IRA-400 (OH)). The column was washed with water until free of reducing sugars. The adsorbed acids were then eluted with 0.1 *N* NaOH. The eluate was evaporated to dryness at room temperature and under reduced pressure.

Experimental Results

Oxygen Consumption of Larvae

Figure 1 illustrates the respiratory pattern of larvae on royal jelly and worker diet of two ages (worker jelly and modified jelly). The pattern of oxygen uptake is similar for larvae on all substrates. In each case the slope of the curve increases when the larvae are from 12 to 14 hours old indicating the time of the first molt. This increase in slope can also be inferred from a comparison of oxygen uptake for the 300-minute and 800-minute periods in Table I.

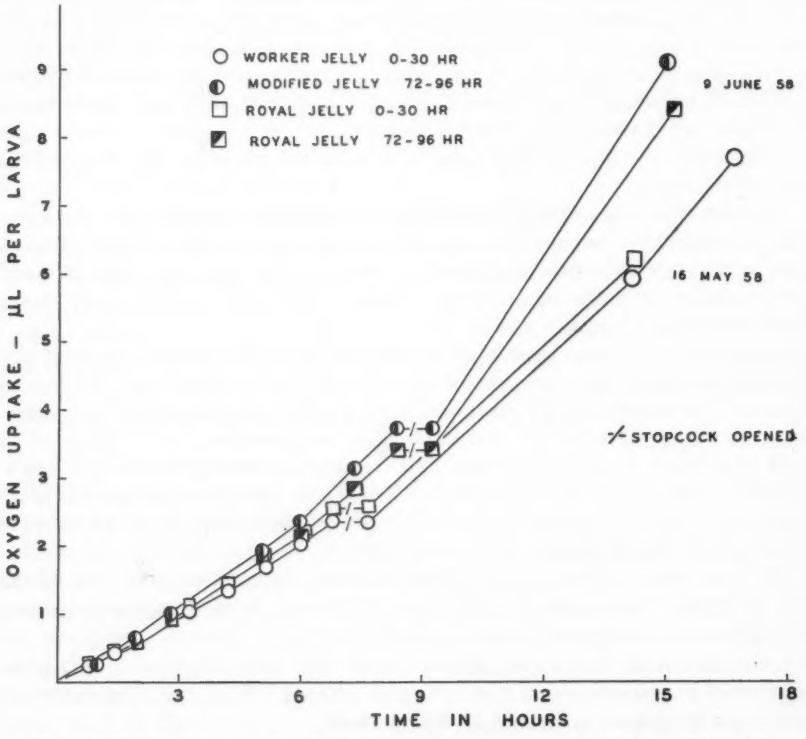

FIG. 1. Pattern of oxygen uptake by honeybee larvae on worker jelly, modified jelly, and young and old royal jelly. Zero time represents 7 hours for larvae of May 16, 4 hours for larvae of June 9.

TABLE I

Oxygen uptake by honeybee larvae on six substrates

Type and age of substrate		Average O_2 consumption per larva (μl) for: 300 min	800 min
Worker diet			
Worker jelly	0–30 hours	1.68±0.102*	5.93±0.465
Worker jelly	40–60 hours	2.01	7.14
Modified jelly	72–96 hours	1.79	7.49
Royal jelly			
	0–30 hours	1.70±0.102	6.80±0.465
	40–60 hours	1.91	7.46
	72–96 hours	1.72	6.86
Average for worker diet of all ages		1.83±0.059	6.85±0.269
Average for royal jelly of all ages		1.78±0.059	7.02±0.269
Average for worker diet and royal jelly aged			
	0–30 hours	1.69±0.072	6.37±0.329
	40–60 hours	1.96	7.30
	72–96 hours	1.76	7.17
Values of *F* for:			
	age	3.77*	2.48
	type	0.47	0.25
	age × type	0.19	1.38

*Standard error.
*Significant at 5% level.

Table I shows the mean oxygen uptake per larva for the first 300-minute and 800-minute periods on six substrates. These data indicate a comparable order of oxygen uptake on worker diet and royal jelly. Mean oxygen uptake values for 40- to 60-hour substrates were slightly higher than for the other ages, a difference which we attributed to seasonal variation in larval vigor, since similar differences were found in measurements of egg respiration (authors' unpublished data).

Carbon Dioxide Evolution by Larvae

The striking feature of Table II is that larvae on both worker jelly substrates showed a slightly negative net carbon dioxide evolution. On all other substrates a large positive net carbon dioxide evolution was manifest.

Results of Microchemical Analyses

Since the work of von Planta (11) analyses on the composition of royal jelly have been reported (5, 6, 19) but a dearth of comparative data on royal jelly and worker diet made further analysis necessary.

Microchemical analyses of the major constituents of the various substrates are shown in Table III. Not shown in the table are corroborative data obtained from other samples. The following observations were made:

1. The reducing sugar content of worker diet increased with age, owing to the addition of honey around the third day of larval life (13). The sugar content of royal jelly, however, remained constant. It was intermediate between that of worker jelly and modified jelly.

TABLE II

Net carbon dioxide evolution by newly emerged honeybee larvae on five substrates

Group comparison		Number of larvae	Mean net CO_2 evolution per larva (μl/1000 min)	Value of t	Value of P*
Worker jelly	(0–30 hours)	14	-0.06 ± 0.059[a]	1.50	>0.1
Worker jelly	(40–60 hours)	20	-0.20 ± 0.063		
Worker jelly	(0–30 hours)	14	-0.06 ± 0.059	6.31	<0.001
Modified jelly	(72–96 hours)	20	$+0.78 \pm 0.094$		
Worker jelly	(40–60 hours)	20	-0.20 ± 0.063	8.69	<0.001
Modified jelly	(72–96 hours)	20	$+0.78 \pm 0.094$		
Royal jelly	(0–30 hours)	11	$+0.88 \pm 0.139$	6.77	<0.001
Worker jelly	(0–30 hours)	14	-0.06 ± 0.059		
Royal jelly	(0–30 hours)	11	$+0.88 \pm 0.139$	0.23	>0.5
Royal jelly	(72–96 hours)	11	$+0.92 \pm 0.092$		
Royal jelly	(72–96 hours)	11	$+0.92 \pm 0.092$	0.96	>0.3
Modified jelly	(72–96 hours)	20	$+0.78 \pm 0.094$		

[a]Standard error.
*Probability of a similar effect due to chance.

2. The protein content of worker diet decreased with age. The protein content of royal jelly remained constant, and was intermediate between that of worker jelly and modified jelly.
3. The ether-extractable fraction of worker diet decreased with age. The weight of the ether extract of royal jelly was relatively constant and was much higher than in worker diet of any age. The titratable acidity of the ether-soluble fraction of worker diet decreased sharply with age. The average values for royal jelly and worker jelly were of the same order of magnitude. The saponification procedure caused no increase in titration values, indicating the absence of a significant amount of saponifiable material.
4. The titratable acidity of the water-soluble material remaining after ether extraction was slightly higher in worker jelly.
5. The titratable acidity of the water suspension of untreated modified jelly was 38 to 47% lower than that of the other diets.

There was relatively little variation in composition of royal jelly fed to young and older larvae. Since royal jelly of all ages is entirely a secretion of the pharyngeal glands of nurse bees (13) it is not unexpected that it should show uniformity in chemical composition. The composition of worker diet does not remain constant. While additions of pollen around the third day of larval life appear to be relatively unimportant (15), the addition of honey has an important dilution effect on the other constituents. This addition is sufficient to account for the observed decreases in protein and titratable acidity of the untreated jelly and the water-soluble residue, but not for the large reduction in weight and titratable acidity of the ether extract (see below).

Kestner (7) reported similar changes in the protein, reducing sugar, and lipid contents of worker diet with age.

TABLE III

Comparative microchemical analyses of larval diets

Type and age of diet	Protein (% dry wt.)	Reducing substances (% dry wt.)	Ether extract (% dry wt.)	Titratable acidity (μeq/g dry wt.)		
				Ether extract	Water-soluble residue	Untreated jelly
Worker jelly (0–30 hours)	49.2±1.03[a]	12.0±1.29	5.2±0.65	347±9.8	649±27.8	1546±29.2
Modified jelly (72–96 hours)	25.3±1.03	47.4±1.29	1.6±0.65	86±9.8	487±27.8	861±29.2
Royal jelly (0–30 hours)	37.3±1.03	33.5±1.29	15.3±0.65	273±9.8	519±27.8	1394±29.2
Royal jelly (72–96 hours)	33.3±1.03	34.2±1.29	13.4±0.65	331±9.8	450±27.8	1633±29.2
Number of analyses included in each mean	5	6	3	6	8	5

[a]Standard error.

The most significant feature of the data in Table III is the difference in chemical composition between the two pure secretions, worker jelly and royal jelly.

Two apparent discrepancies may be noted in the analytical data: (i) The combined values for the titratable acidities of the ether-soluble and water-soluble fractions are less than the total titratable acidity values for untreated jelly, and (ii) although the weight of the ether extract is much higher in royal jelly than in worker jelly, the titration values of the respective ether extracts are comparable.

Carbon Dioxide Evolution on Artificially Altered Diets

Dietary changes could account for the characteristic pattern of carbon dioxide evolution. Carbon dioxide evolution of larvae on diets altered artificially was next measured.

The first experiment was designed to simulate the natural change in sugar content of the worker diet. Fructose and glucose in equal amounts were added to worker jelly, increasing the total solid content from 29 to 39%. The sugar addition increased the net carbon dioxide evolution to a level approximately midway between that of worker jelly and modified jelly (Table IV, comparisons 1 and 2). Thus the large change in the sugars in the natural diet does not account for all of the increase in carbon dioxide.

The second experiment was designed to study the effect of increasing the ether-soluble acidity of modified jelly to equal that of worker jelly. Royal

TABLE IV

Comparisons of net carbon dioxide evolution by newly emerged honeybee larvae on natural and artificially altered substrates

Group comparison	Number of larvae	Mean net CO_2 evolution per larva (μl/1000 min)	Value of t	Value of P*
1. Worker jelly (0–30 hours)	8	-0.19 ± 0.081[a]	3.77	<0.01
Worker jelly (0–30 hours) + reducing sugars	11	$+0.44 \pm 0.129$		
2. Modified jelly (72–96 hours)	9	$+1.29 \pm 0.065$	11.64	<0.001
Worker jelly (0–30 hours) + reducing sugars	10	$+0.64 \pm 0.078$		
3. Modified jelly (72–96 hours)	8	$+1.09 \pm 0.108$	0.75	>0.5
Modified jelly (72–96 hours) + ether extract	7	$+1.18 \pm 0.097$		
4. Modified jelly (72–96 hours)	6	$+1.26 \pm 0.074$	18.00	<0.001
Worker jelly (0–30 hours) + reducing sugars + water-soluble acids	9	$+0.06 \pm 0.026$		
5. Worker jelly (0–30 hours) + reducing sugars	21	$+0.53 \pm 0.077$	3.98	<0.001
Worker jelly (0–30 hours) + reducing sugars + water-soluble acids	9	$+0.06 \pm 0.026$		

[a]Standard error.
*Probability of a similar effect due to chance.

jelly, which is obtainable in greater quantity than worker jelly, was used as a source of the added acids. A quantity of the ether extract of lyophilized royal jelly, sufficient to give the titration value noted in Table III for worker jelly (approximately the same as that of royal jelly), was added to a sample of modified jelly. The total solids were thereby increased from 36 to 38%. Except for its lower protein content, this artificial diet was very similar to royal jelly in its gross composition. Carbon dioxide evolution on this diet was comparable to that noted for modified jelly (comparison 3, Table IV), and, by analogy, for royal jelly (Table II).

The final experiment dealt with the addition of water-soluble acids. A sufficient quantity was added to the artificial diet containing extra sugar, to increase its water-soluble acidity to 1000 microequivalents per gram dry weight. This addition changed the total solids from 39 to 41%. The effect of the added water-soluble acids on respiration is seen in comparison 5, Table IV. Net carbon dioxide output was reduced to the extent of nullifying the increase caused by the added sugars.

Larvae cultured on all artificially altered substrates were normal in size and appearance. The only irregularity noticed was the appearance of abdominal peristaltic waves which seemed to accompany normal spiracular action in larvae on the diet containing added water-soluble acids.

Discussion and Conclusions

The data on oxygen consumption apparently do not provide a basis for suspecting any difference in larval respiration between royal jelly and worker diet. The differences in carbon dioxide evolution on worker jelly and royal jelly substrates support the validity of using respiration as an early measurable criterion of sexual dimorphism in the larval honeybee. During the first 24 hours of larval life the rate of carbon dioxide evolution is higher in queen larvae than in worker larvae. Moreover, the compositions of the respective secretions differ. Further work is necessary on the elaboration of these secretions by the pharyngeal glands. There is a difference of opinion as to whether the secretions are elaborated by the same group of nurse bees at different times, or by different groups of nurse bees (8, 12). This problem should be approached on both cellular and population levels. Such studies might explain why addition of honey to worker jelly does not completely account for all the changes observed in the chemical composition of worker diet.

The data suggest that the extrinsic factor of nutrition is responsible for the characteristic respiratory patterns. The experiments reported in Table IV, although not exhaustive, demonstrate that these patterns are amenable to change. Modified jelly, which differs markedly from worker jelly by reason of an increase in reducing sugars of approximately 300%, supports a level of carbon dioxide evolution similar to that of royal jelly. Although fortifying worker jelly with reducing sugars increased the carbon dioxide production, it did not raise it to the level observed for modified jelly. The effect of added

sugar was reversed by an addition of the water-soluble acid fraction of royal jelly. The striking effect of this latter addition, small by comparison with the sugar addition, was unexpected. By contrast, raising the ether-soluble acid content of modified jelly, which was very low, had no significant effect on carbon dioxide evolution.

Varying the ratio of major dietary components can alter the net carbon dioxide, and hence the respiratory quotient. Caution, however, should be exercised in attributing changes in respiratory quotients to a specific component. Melampy and Willis (9) obtained respiratory quotients of 1.16 and 1.42 for 2- to 3-day queen and worker larvae, respectively. They attributed the R.Q.'s above unity to lipid synthesis. Their data of a subsequent paper (10), however, showed that fat synthesis occurred at a faster rate in queens than in workers.

Female dimorphism in the honeybee is a function of nutrition (13) and is manifest in different respiratory patterns (9). The present study has established a relationship between diet and respiration during the first day of larval life. An hypothesis of nutritional balance could account for these correlations. Early differences in larval metabolism probably have biological significance in differentiation. It seems probable that differentiation begins during the first day of larval life although it may remain susceptible to modulation until the third day (13). Although a critical test of the hypothesis must await the development of a reliable bio-assay the results of experiments performed to date on the early feeding of female honeybee larvae are consistent with an hypothesis of nutritional balance. Larvae which are fed for 1 to 3 days on worker jelly and then transferred to royal jelly produce some queens, some intermediate types, and some workers (13, 17, 21).

The ultimate effect of nutritional balance on form is probably exerted via hormonal action. The concept of the relationship between nutritional balance and sexuality has been developed by Salt (14). The variety of adult female morphological characteristics including ovariole development (17, 21) is probably the result of changes in hormonal balance at critical times in the differentiation of imaginal characteristics within the young larvae. Partial sexual expression, i.e. the development of ovaries which are functional, though the number of ovarioles is reduced, is possible even in adult workers (13).

Lack of sexual maturity is a juvenile characteristic; in other words the ovaries have failed to "realize" their potentialities because the hormonal balance is different from that in developing queen larvae. Waddington (20) has pointed out with respect to insect hormones that "they act. . . as 'realizers', which make it possible for potentialities to become actual, but they are not 'determiners' which could change the character of reacting tissues."

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NOTES

RECORD OF WYOMINIA TETONI SCOTT, 1941 FROM OVIS DALLI IN THE YUKON TERRITORY

H. C. GIBBS[1] AND W. A. FULLER[2]

Specimens of *Wyominia tetoni* were collected from four male *Ovis dalli* in 1955 and 1958 from Sheep Mountain at the mouth of Slim's River, which is mile post 1061 on the Alaska Highway (61° 00′ N., 138° 35′ W.). This is a new host and locality record for this species, which has previously been reported from Wyoming in *Ovis canadensis canadensis* by Scott (1), Honess and Frost (3), and Honess and Winter (4), and Idaho by Smith (2).

The sheep ranged in age from 18 month to 7½ years and were all parasitized. The yearling carried a single worm, while the adults had approximately 10 worms each.

The over-all condition of the sheep was good to excellent. The worms were found with the anterior ends in the bile or hepatic ducts. They filled the bile duct, and the posterior ends protruded into the lumen of the small intestine. They apparently caused little damage to the host tissues except for local thickening and hypertrophy of the bile ducts. Honess and Winter (4) claim that chronic infections with this species result in the enlargement of the bile duct and replacement of the epithelium with granulomatous tissue. This interferes with the flow of bile and produces a chronic icteric condition.

Big game guides when questioned on the prevalence of this parasite could not recollect having seen it in sheep which is remarkable since they regularly eat sheep livers. However, one resident mentioned that she had seen it in sheep from the Donjek River area, about 40 or 50 miles northwest of Slim's River.

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